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OPHTHALMOLOGY

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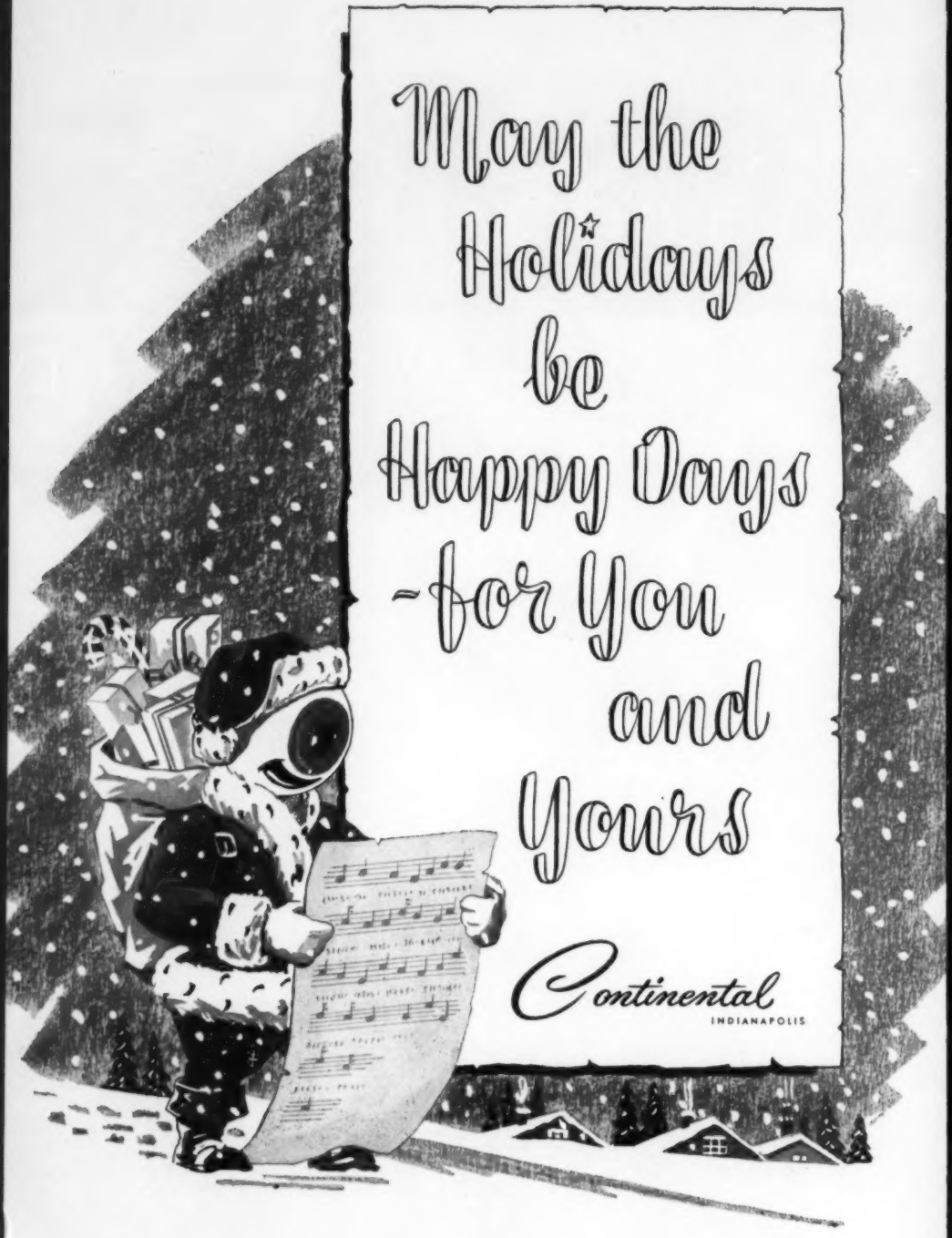
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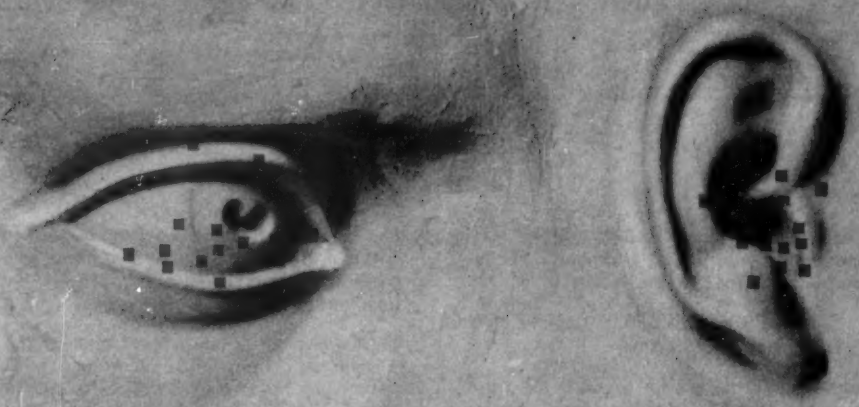

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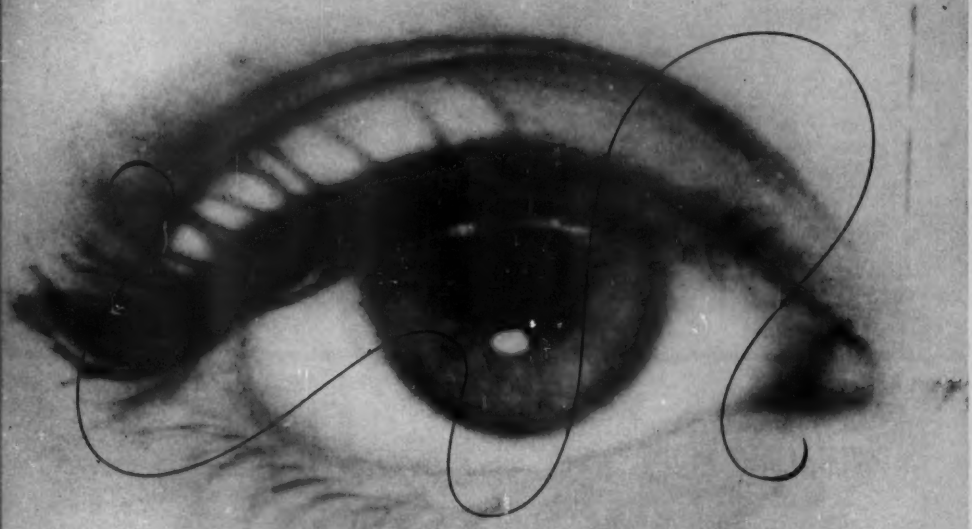

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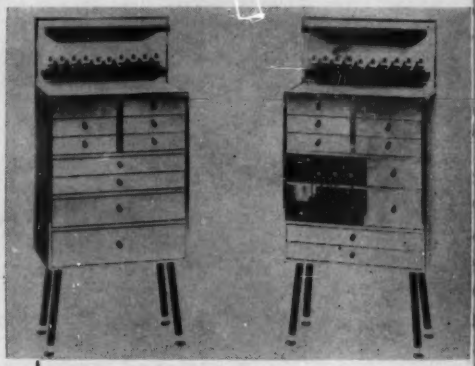

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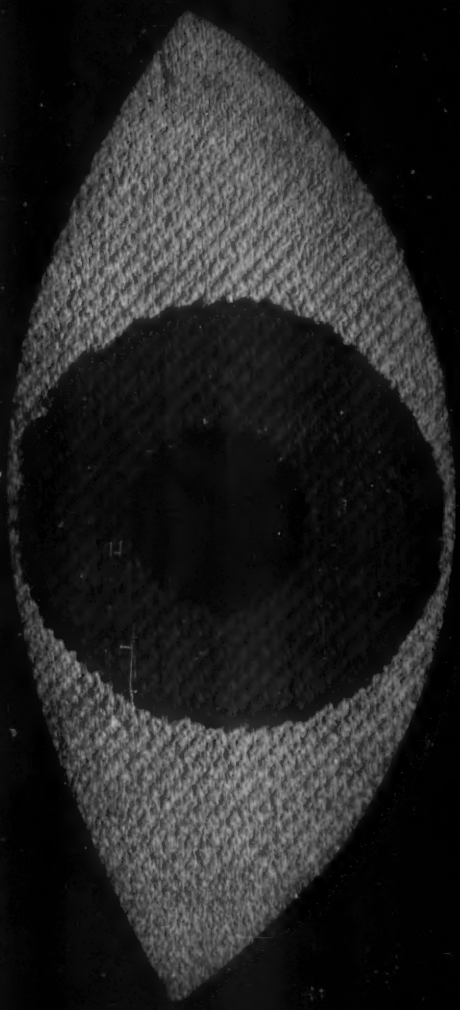

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References: (1) Perkins, E.-S. **Practitioner** 178:575, 1957. (2) Smith, C. H. **Eye, Ear, Nose & Throat Month.** 34:580, 1955. (3) Blakiston's New Gould Medical Dictionary, ed. 2. New York, McGraw-Hill Book Company, Inc., 1956, p. 945. (4) Cutler, H. B. & Braley, A. E. **J. Iowa M. Soc.** 44:477, 1954.

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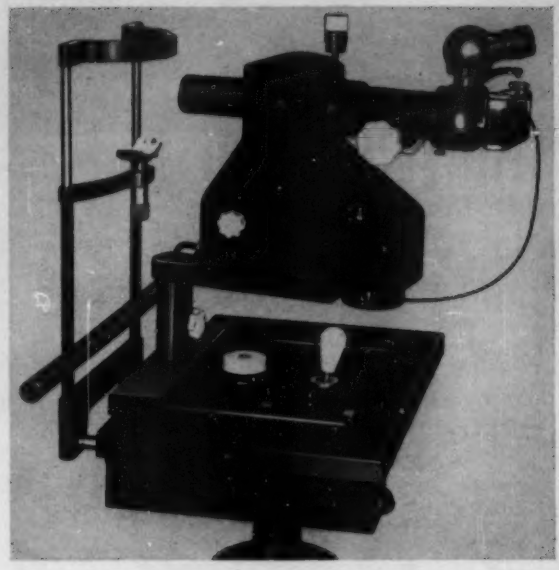

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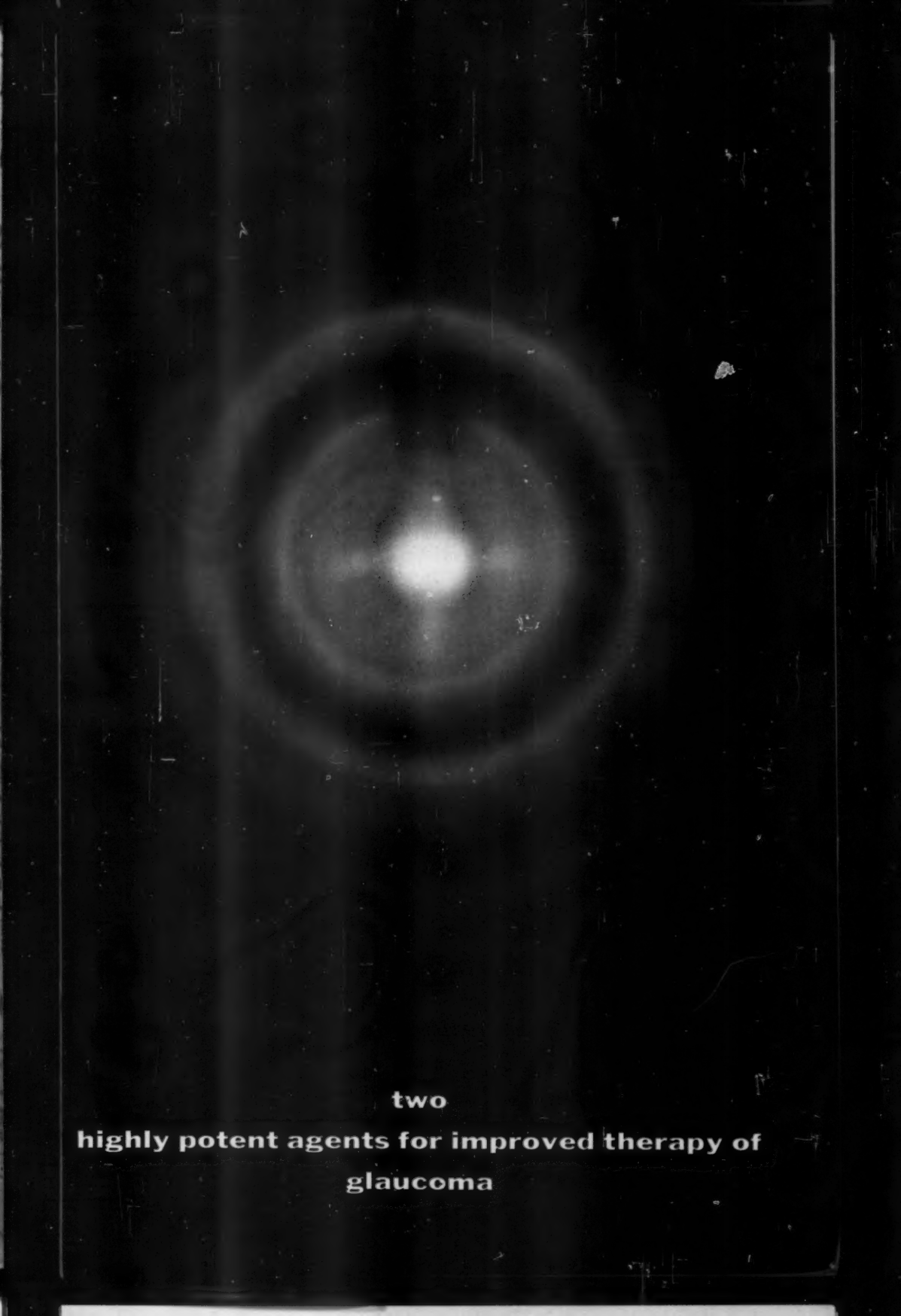
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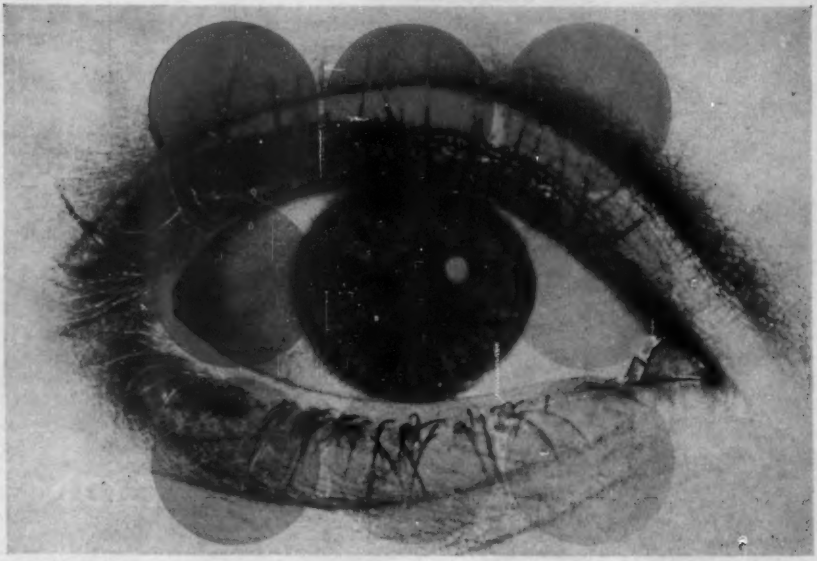

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1. New and Nonofficial Drugs; J. B. Lippincott Company, Philadelphia, 1958, p. 248.

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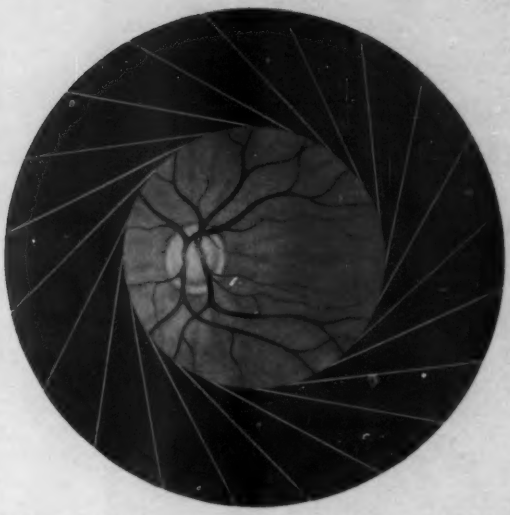

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1. Priestly, B. S.; Medine, M. M., and Phillips, C. C. To be published. 2. Ahlquist, R. P. in Drill, V. A.: Pharmacology in Medicine, McGraw-Hill Book Company, Inc., New York, 1954, p. 18-55.

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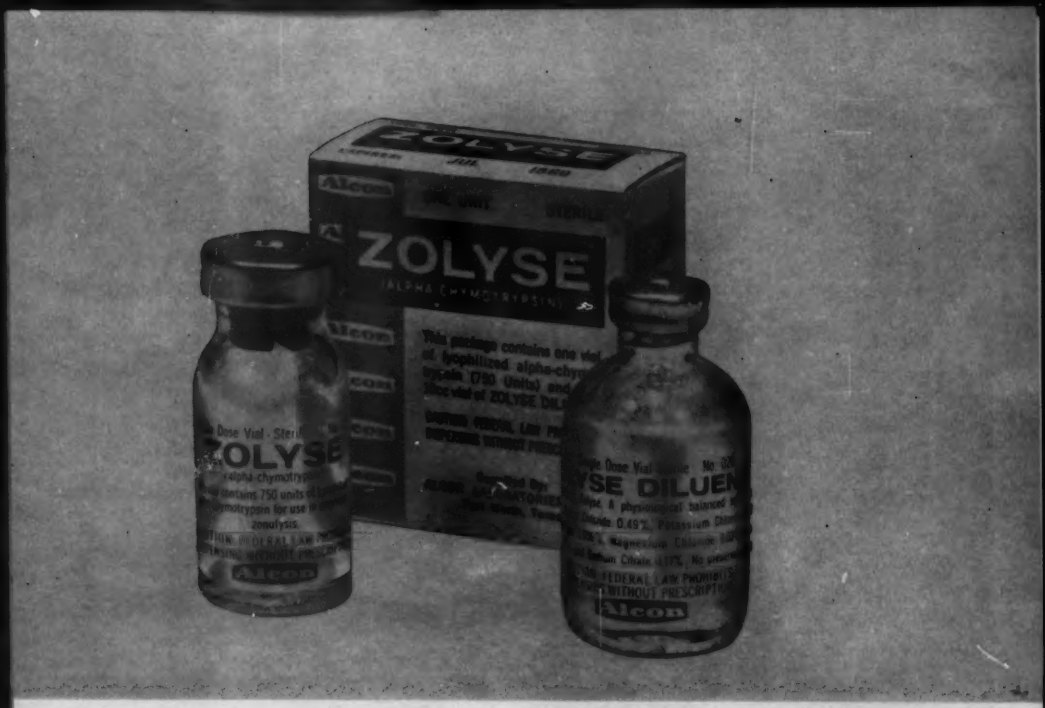

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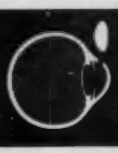
[1] Girard, L. J., Dukes, C. D., and Fleming, T. C. Presented at the International Congress of Ophth., Brussels, Belgium, 1958.
[2] Kara, Gerald D., "The Use of Alpha-Chymotrypsin in Cataract Extraction," Research Report No. 16, Alcon Laboratories, Inc., 1959.
[3] Vail, D., et. al. Report: Committee on use of alpha-chymotrypsin in ophthalmology. Presented at the Sixty-fourth Annual Session, American Academy of Ophthalmology and Otolaryngology, Chicago, Oct. 11-16, 1959. (To be published)



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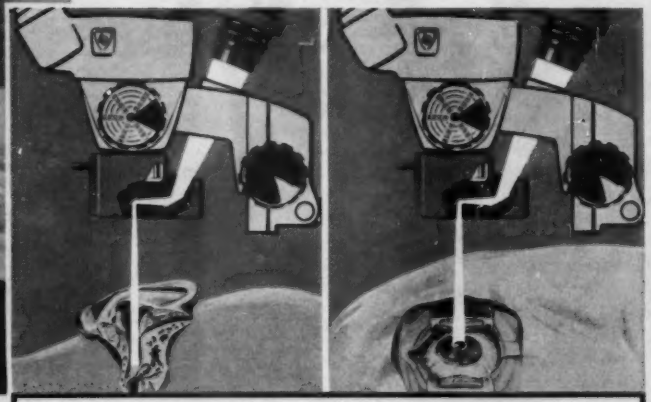

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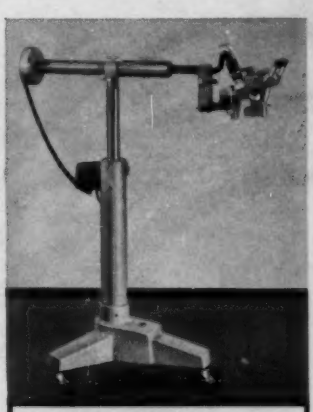

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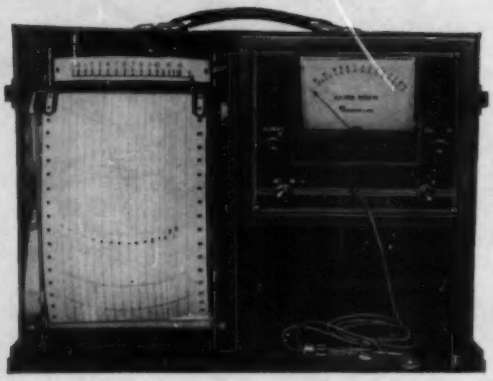

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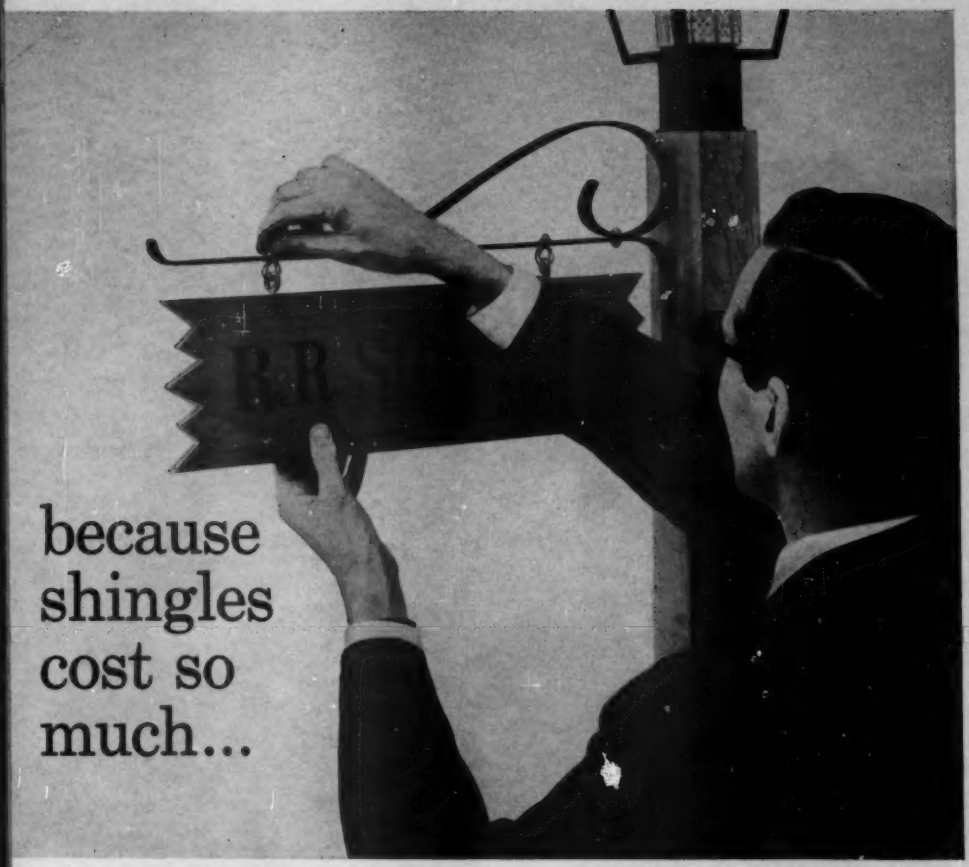

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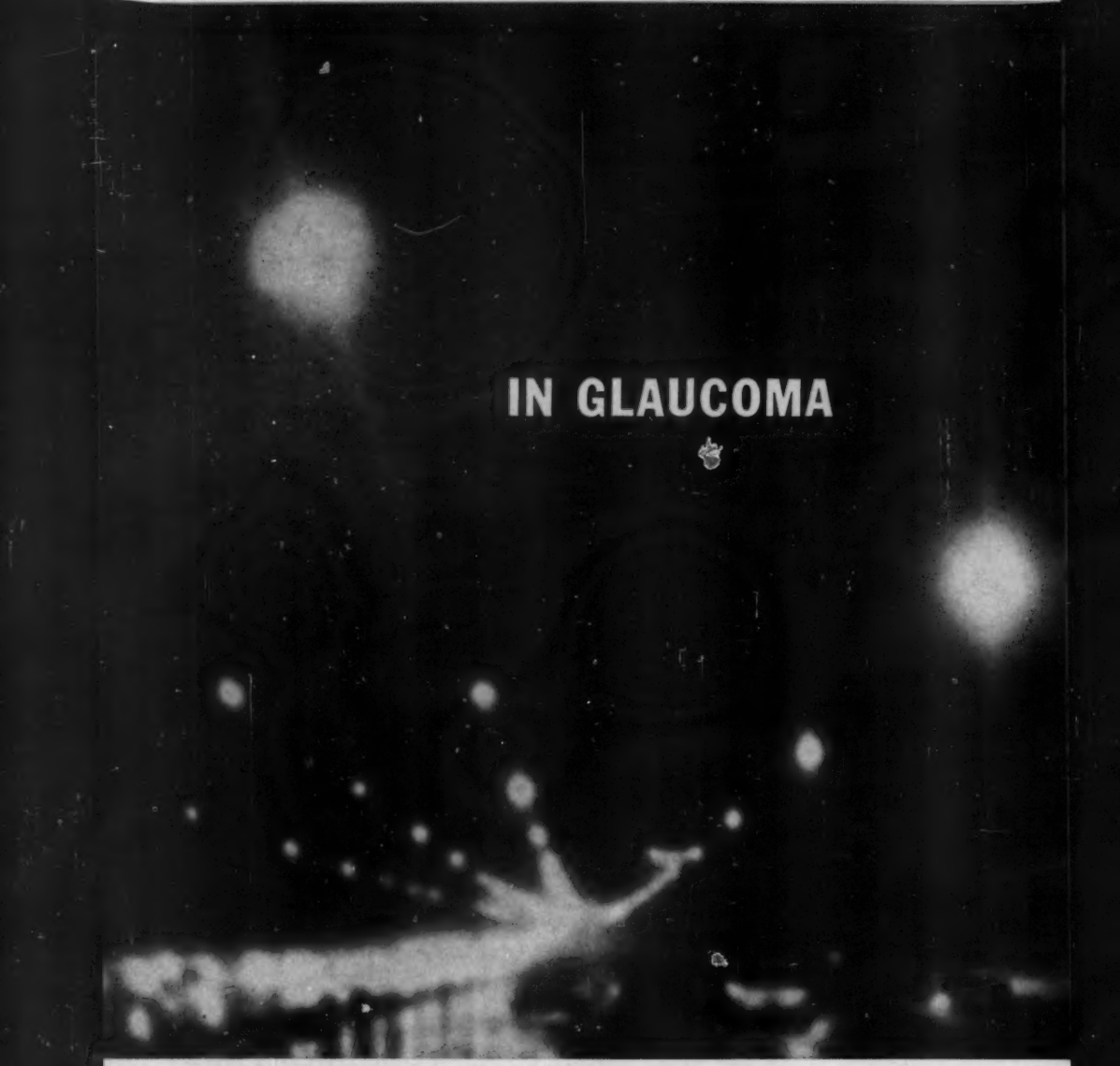

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Some General Principles of Electron Microscopy

BEN S. FINE, M.D.; A. J. TOUSIMIS, and LORENZ E. ZIMMERMAN, M.D., Washington, D.C.

Throughout this issue of the ARCHIVES the reader will find the results, mainly in the form of micrographs, of morphologic studies of ocular tissues obtained with the electron microscope. The techniques employed in electron microscopy differ considerably from those of light microscopy. For the proper appreciation of these results obtained with this instrument some knowledge of the methods used in specimen preparation is required. The purpose of this presentation is to outline some of the principles, materials, and techniques of electron microscopy.

The resolution of the average human eye (i.e., the closest two points or lines can be together and still be perceived as separate) is considered to fall in the range of 0.1-0.2 mm. (100μ-200μ). This inherent limitation of the eye is overcome by use of magnifying lenses which refract light to "spread" these two points apart, so that intermediate points or lines may be distinguished as separate. Glass lenses, together with visible light, enable the observer to distinguish two points or lines 2,500 A. (0.25μ) apart.* This results in maximum useful magnifications of about 2,000 diameters. The limitation in resolving power of glass lenses rests not in their imperfections, most of which have been overcome, but in the nature of light itself. The resolving power improves as the wave length of the light employed decreases. The ultraviolet microscope makes use of shorter light waves to attain resolutions of the order of 1,000 A., more than doubling the magnification of the ordinary light microscope. By use of the shorter wave lengths of the electromagnetic spectrum (i.e., the x-rays), it should be possible to attain better resolutions. Unfortunately, however, x-rays cannot be used in conventional optical systems because these rays cannot be focused by glass, quartz, magnetic, or electrostatic lenses.

The Electron Microscope

The development of high resolution microscopy relies upon the use of another type of radiation. The electrons which behave both as particles and as waves (i.e., similar to the dual nature of electromagnetic radiation) have been found suitable. Electron beams (cathode rays) have very short wavelengths (of the order of 0.05 A. for 50 kv. electrons) and can be deviated by

The halftone illustrations of this article will be found in the special section of illustrations, pages 1001 and 1002.

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From the Ophthalmic Pathology Branch, Armed Forces Institute of Pathology.

Special trainee of the National Institute of Neurological Disease and Blindness, National Institutes of Health (Dr. Fine).

*1 cm. 10 mm.
1 mm. 1,000 microns (μ)
1μ 1,000 millimicrons (mμ)
1 mμ 10 angstroms (A.)

Range of visible light perceived by the normal human eye 4,000-7,000 A.

an electrostatic or magnetic field. A properly shaped magnetic field (created usually by electromagnets) is, therefore, used as a lens to focus a beam of electrons in much the same way as a glass lens bends and focusses a beam of light.

The resolution of a modern electron microscope is of the order of 10 A. (0.001μ), a gain by a factor of 250 or more in resolving power and useful magnification when compared with the light microscope. The geometric optics of this instrument and even the physical arrangement of its parts in the microscope column resemble those of the light microscope (Fig. 1). The light rays, which in the conventional microscope enter the bottom of the column, are replaced by a beam of electrons (cathode rays) emanating from a heated filament (usually tungsten) placed at the top of the microscope column. Below this source of electrons in descending arrangement is a series of circular electromagnets, the lenses, the beam of electrons passing through their central apertures. These lenses are given analogous names (viz., condenser lens, objective lens), but the "ocular" is called "projector" for it projects the final image onto a fluorescent screen for viewing. The beam of electrons can be focussed by altering the amount of current flowing through these electromagnetic lenses. For proper operation this entire column must be evacuated to the level of vacuum found in a good television tube ($10^{-4}-10^{-5}$ mm. Hg). The remainder of the instrument consists of a high-voltage power supply to provide an extremely constant accelerating potential† for the electron beam, current controls for the magnetic lenses, a high capacity pumping system to maintain or renew the vacuum each time a new specimen is to be examined, and a system for recording the images of photographic plates.

As in light microscopy, the specimen is placed on the condenser side of the objective lens in the microscope column. Because of the vacuum in the column chamber where the specimen is placed, examination of wet or living tissue is not practical.

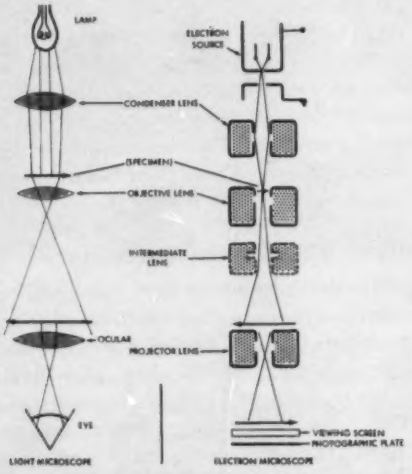

Fig. 1.—The arrangement of the component parts in the light microscope, shown inverted on the left, is analogous to that of the electron microscope on the right. The illumination in the conventional microscope is visible light, while in the electron microscope the specimen is "illuminated" by electrons. The "lenses" in the electron microscope are electromagnets, and the entire assembly including the specimen is in vacuum.

Materials and Methods

Because of the use of electron beams instead of light, and because of the necessity for examination in vacuum, special methods are employed in the preparation of specimens for suitable examination.

A. Fixation.—Most of the fixatives used in light microscopy are not suitable for electron microscopy. Suitably buffered fixatives containing heavy atoms, such as are present in osmium tetroxide and potassium permanganate are widely used. These compounds function as fixatives and at the same time, because of their high electron scattering power, increase the contrast of the specimen. Unfortunately they penetrate tissues very slowly, so that the latter must be fixed in very small pieces (1 mm. thick). In addition, it is essential that the tissue be placed in the fixative within the shortest

† The focusing of the beam of electrons depends both on their velocities (determined by accelerating potential) and magnetic field of the lens.

possible time (10 min.) after their removal, in order to minimize autolytic effects on its ultrastructure.

B. Embedding.—Suitably prepared tissue is dehydrated in graded alcohols (ethyl or methyl) or acetone. The tissue is finally impregnated by liquid plastic (e.g., monomer of methacrylate) which is subsequently polymerized to suitable hardness.

C. Sectioning.—The magnified image of the specimen is produced mostly by the scattering of incident electrons. Because of this there is for most electron microscopes in use today an optimum thickness of tissue which may be examined without loss of contrast. This thickness is of the order of 0.050μ (Figs. 2 and 3), in contrast to the sections prepared for light microscopy which are of the order of 5 to 10 microns. To cut such thin sections, the plastic embedded tissue is firmly mounted in a specially designed microtome (ultramicrotome) which advances the specimen as little as 0.025μ with each revolution.

Although specially sharpened steel knives or razor blades are used to cut these extremely thin sections, the glass knife is by far the most widely used of all the cutting tools. This consists of a piece of ordinary plate glass broken in such a way as to produce a sharp, but very short edge for cutting (less than ¼ inch).

D. Mounting.—Instead of a glass slide, the specimen is mounted on a very thin film (100-200 A. thick) supported by a small copper screen or "grid". These films are usually made of parlodion, formvar, or carbon. Such films are, for all practical purposes, transparent to the electrons and perform the same function as a glass slide in light microscopy.

E. Particulate Matter.—Very small particulate matter such as dust, bacteria, viruses, or connective tissue elements may be examined by drying suspensions of these materials on the plastic covered copper screen and examining them directly. Detail within these particles can be observed provided that a variation of densities is present. Tissues fractionated by homogenization and differential centrifugation (e.g., mitochondria, microsomes, pigment granules, collagen, and other components) can be examined with the electron microscope. These materials can be examined as suspensions or after embedding and sectioning.

F. Shadowcasting.—This technique consists of evaporating a heavy metal (chromium, palladium, or uranium) in a vacuum chamber, so that the atoms of metal which travel in all directions in straight lines deposit themselves over the specimen and its support (Fig. 4). The portion of the supporting film lying shielded by the specimen receives no metallic covering and, therefore, in subsequent examination in the electron microscope, permits passage of more electrons. This results in a brighter image on the viewing screen which will appear as a shadow on the photographic plate. This

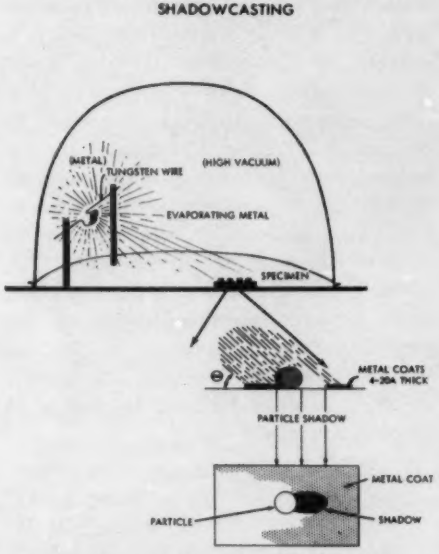

Fig. 4.—The metal used for shadowcasting is evaporated onto the specimen in a vacuum chamber shown in the upper part of the diagram. The angle of shadowing (θ) is determined in advance; thus the height of the particle (center of diagram) can be calculated from shadow length measurement. The final electron micrograph, resulting from such a preparation is shown in the lower part of the figure. More electrons pass through the portion which has no metal coating, resulting in a shadow.

method produces a three dimensional effect and enhances the contrast of a specimen enabling the determination of size and shape of small particles (Fig. 5). Shadowcasting is reminiscent of the methods employed by aerial photographers who take pictures with a low sun, so that the shadows cast will bring out the small projections on the surface of the earth in strong relief. Ophthalmologists use a similar technique when they apply what is called indirect, lateral, or oblique illumination with the slit lamp to make objects adjacent to the illuminated surface stand out in relief.

G. Shadowed Tissue Sections.—Mention has been made of the methods used to obtain very thin sections of tissue suitable for examination in the electron microscope. However, in this effort to obtain thinner and thinner sections, information concerning materials which are usually found in lower concentration than within cells (i.e., intercellular substances) becomes unobtainable. One way to overcome this loss is to examine thicker sections (0.10μ or more) of tissue with the sacrifice of detail of intracellular structure. In order to increase the contrast and stabilize the specimen, the embedding medium is dissolved away and the remaining tissue structures are then shadowcast. This method has been used in the study of both plant and animal tissues. Elsewhere in this issue there appears a paper which is based upon application of this method to the examination of the iris stroma. This tissue is emminently suitable for this type of examination because of its peculiar structure.

H. Replicas.—Examination of the surface of a solid is accomplished by replica methods. Replication methods have been used in light microscopy of ocular tissue but not yet for electron microscopy. In electron microscopy this consists usually of making a thin plastic cast of the surface. This is then stripped off and shadowcast with a heavy metal; thus upon examination in the electron microscope, the irregularities of the surface will stand out in bold relief.

I. Others.—A number of other techniques and methods of tissue treatment are available or are in the process of development. These are somewhat like the special stains of ordinary histology and are employed to obtain answers to specific questions. Stereoscopic viewing, electron diffraction, use of other embedding media such as resins (araldite), and cutting with the diamond knife all find their areas of usefulness. Certain biochemical and histochemical techniques may also be applied to tissues which are subsequently examined in the electron microscope.

Summary

Electron microscopy, as it is widely practiced today, employs a beam of electrons, a series of electromagnetic lenses, and the principles of geometric optics to achieve much greater resolution (and useful magnification) than is possible by light microscopy. A variety of materials and methods have been developed to apply this type of microscopy to the study of animal tissues. Some of the principles behind these techniques are reviewed.

Armed Forces Institute of Pathology (25).

Ultrastructure of the Ciliary Epithelium

AKE HOLMBERG, M.D., St. Louis

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A. Introduction

Since the publication of the analysis of the ultrastructure of normal and acetazolamide (Diamox)-treated nonpigmented ciliary epithelium in the rabbit eye (Holmberg, 1957), the investigation has been extended to include the pigment epithelium in adult rabbit and both epithelial layers in monkey, human, and young rabbit eyes. The ultrastructure of the ciliary body capillaries and of the external and internal limiting membranes have been described on pages 949-951 in this issue and will be omitted from this presentation.

Because of the limited resolution of the light microscope, very little has been added to Seidel's extensive work during the second and third decade of this century on the morphology of the ciliary epithelium and its alterations. The electron microscope has made it possible to examine tissues on a macromolecular level and to define a large number of cellular components, previously not known. In some cells it has also been possible to correlate submicroscopic constituents to special cellular functions.

The present analysis of the fine structure of the ciliary epithelium concerns the findings in normal cells and the changes induced experimentally.

B. Materials and Methods

For evaluating the normal structure of the ciliary epithelium in different species, the following material was used: albino and pigmented rabbits weighing 2-3 kg., young rabbits of ages between 1 and 30 days, young monkeys, and two fresh human eyes, 30-40 years of age.

The experiments with acetazolamide and controls were performed on albino and pigmented rabbits weighing 2-3 kg. All animals were nephrectomized 15-20 hours before the experiments started. The dosage of acetazolamide was 10 mg. per kilogram of body weight given intravenously in a 1%

The halftone illustrations for this article will be found in the special section of illustrations, pp. 1003 to 1032.

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solution. As a control substance Diazil was used in the same amount and concentration. The period of treatment with acetazolamide or Diazil varied from 15 minutes to 2 hours.

One patient with a unilateral malignant melanoma was given 500 mg. of acetazolamide intravenously 15 minutes before enucleation.

The fixation, dehydration, and embedding technique used in this work were the same as are routinely used in electron microscopy with small modifications. The fixative consisted of 1% osmium tetroxide solution in barbital (Veronal)-acetate buffer (Palade, 1952) brought to isotonicity according to Sjöstrand (1953) by adding sodium, potassium, and calcium chloride. For increasing the contrast in the tissue 0.1% phosphotungstic acid was used in 70% alcohol during the dehydration procedure. The tissue was embedded in a methacrylate mixture according to Newman, Borysko, and Swerdlow (1949). Sectioning was performed partly on the Sjöstrand Ultramicrotome and partly on the Porter-Blum microtome. The sections were examined in RCA EMU 2C and 2TE microscopes.

C. General Topography

The material studied was taken exclusively from the ciliary corona, where the ciliary processes are very pronounced. Each process presents a central core of stroma and capillaries, covered by a double layer of epithelium, a nonpigmented layer bordering on the posterior chamber and an outer pigmented layer. Between the stroma and the pigmented layer there is an external limiting membrane and between the posterior chamber and the nonpigmented epithelium there is an internal limiting membrane. Figures 1 and 2 are light micrographs showing the relationships between different parts of a ciliary process. They are presented here for the purpose of orientation and correlation with the electron microscopic appearance.

The most obvious differences between the two cell layers are the presence of pigment granules in the outer layer and the difference in size of the cells. In the inner nonpigmented layer the cell height is 10μ-15μ as compared to 4μ-6μ in the outer layer. The nonpigmented cells are cubical or cylindrical, while the pigment cells are considerably flatter, so that each cell usually borders on more than one nonpigmented cell. Other differences between the two layers will be considered later. Characteristic of the nonpigmented epithelium are large and deep indentations between adjacent cells, giving the apical part of the cells the form of a cupola.

D. The Normal Structure

1. *Nonpigmented Epithelium.* — *a.* Internal Limiting Membrane: The internal limiting membrane of the rabbit and human ciliary body have been discussed in detail elsewhere in this issue. In the monkey this membrane is much the same as in the rabbit as regards its thickness, electron density, and limitations. In other words, the external limiting membrane in both rabbit and monkey eyes has the appearance of a regular basement membrane (Fig. 7). In contrast to this is the human eye, where the membrane appears as a broad meshwork covering the surface of the nonpigmented epithelium (Fig. 4).

b. The Cell Membrane and β-Cytomembranes: The cell membrane appears in electron micrographs as a comparatively dense, dark layer, about 40 A in thickness.

A characteristic feature of the nonpigmented ciliary epithelium is the presence of β-cytomembranes, in the apical part of the cell close to the cell surface (Figs. 5 and 6). β-Cytomembranes are defined by Sjöstrand (1956) as "membranes which represent tight folds of the cell membrane extending in the interior of the cell body and which do not constitute boundaries facing the surrounding milieu, that is the two membranes of a fold are in close contact." The β-cytomembranes are present in the nonpigmented epithelium of all three species examined and are identical as to

thickness and composition. They represent folds only of that part of the cell membrane which faces the internal limiting membrane, and they are only found in the apical part of the cells. In no case have they been encountered lower than the level of the upper third of the nucleus. Since they do not extend over the entire cell, their frequency varies from section to section. In detail they appear as triple-layered membranes with two osmiophilic layers facing an osmiophobic layer. The total thickness is about 160 A, the opaque layers are about 40 A each, and the less opaque layer is 80 A wide. The membranes are very regular, but occasionally one sees a widening of the osmiophobic layer to form large vacuoles. In no instances and in no species have connections or communications between the β-cytomembranes and other cell components been observed.

Where two adjacent cells border each other the cell membranes come in close contact, forming a double membrane about 160 A wide. The regular less opaque space, which separates the opaque cell membranes indicates, however, that each cell membrane consists of two parts, one osmiophilic and one osmiophobic, each about 40 A wide. The border line between two adjacent cells is not a straight line but is folded extensively so that the cells form long digit-like extensions into each other (Fig. 7). This is especially marked in the apical two-thirds of the cell, and toward the base of the cell the border is straighter. Although all three species examined show the characteristic foldings of the cell membrane at the boundaries, they appear more pronounced in the rabbit eye than in human and monkey eyes.

The boundary line between the nonpigmented and pigmented layer is in principle built in the same way as is the boundary between the nonpigmented cells described above. However, there are very few and small folds or extensions from one cell to another. In addition, this part of the cell boundary has a large number of terminal bars (Fig. 8). These are areas along the cell boundary, where the cytoplasm close to the cell membrane shows a greater density than elsewhere.

c. α-Cytomembranes: The α-cytomembranes are defined by Sjöstrand (1956) as "about 50 A thick membranes to one side of which opaque angular shaped particles with a mean diameter of 150 A are attached. These membranes bound irregularly formed and in most cases very flat spaces." In the nonpigmented ciliary epithelium the α-cytomembranes are scattered throughout the cytoplasm. Occasionally they are found in groups of 10-20 membranes (Fig. 9). In the rabbit eye they are mostly apical to the nucleus, but in human and monkey eyes they are more frequent in the basal part of the cell (Fig. 10). In the ciliary epithelium the α-cytomembranes appear as separate units with no connections between the individual membranes. The space bounded by the membrane, although varying in size, has never been found to reach the configuration of large vacuoles.

d. Golgi Apparatus: There are no reports in the literature about the light microscopic appearance of Golgi apparatus in the ciliary epithelium. It is, however, very easy to localize with the electron microscope. This particular cell component has the same location and composition in all three species examined (Figs. 8, 10, and 11). Without exception it is encountered in the basal part of the cell, in most instances below the nucleus. The Golgi apparatus in the nonpigmented ciliary epithelium consists of a system of paired membranes and a variable number of small vesicles. The membranes lie parallel to each other, and the vesicles are scattered mostly at the periphery of the membranes. The length of the membrane system and the number of vesicles are of the same magnitude in both rabbit, human, and monkey eyes. In the Golgi region of the nonpigmented epithelium large vacuoles are rarely seen.

e. Mitochondria: Mitochondria are seen in all parts of the cell and do not seem to

be more concentrated in any particular region. In the ciliary nonpigmented epithelium they are sausage-shaped bodies with a length of 2μ-3μ. The width varies in the different species. The mitochondria in the rabbit eye have a mean width of 0.18μ, whereas in man the mean width is 0.22μ and in the monkey 0.26μ. The fine structure of the mitochondria is the same in all three species. They have a boundary of a triple-layered membrane and a system of triple-layered membranes in the ground substance. These membranes are arranged parallel with each other and in most cases perpendicular to the long axis of the mitochondria (Fig. 12).

f. Nucleus: The nucleus in the adult species always occupies a large space in the basal part of the cell. It is usually oval-shaped and displays large indentations. The nuclear substance consists of granules, about 150 A in size. The granules are more densely packed at the site of the nucleolus. The nucleus is surrounded by a triple-layered membrane about 200 A thick, in which occasional discontinuities are seen (Fig. 30).

g. Granules: In the nonpigmented epithelium different kinds of granules are seen. In rabbits a varying number of solitary osmiophilic granules are found in the basal part of the cell. These are 0.15μ-0.35μ in size and contain strongly osmiophilic grains in their ground substance (Figs. 8 and 12). In the human material the granules differ in appearance and distribution. They are present in all part of the cells, and they are almost always found in aggregates (Fig. 10). The individual granule is homogenous and the size of each granule varies from 0.05μ to 0.65μ. The nonpigmented ciliary epithelium of the monkey seems to lack granules of this type.

In addition to the type of granules described above, human material contains granules varying from 0.1μ to 0.6μ and without pronounced osmiophilia. These granules have a distinct membrane and homogenous ground substance. They are generally located at the basal part of the cell.

h. Ground Substance: Besides the cell structures described above, there are at least three more components in the nonpigmented epithelium. Small granules, about 150 A in size, are scattered throughout the cell. They appear identical with the granules attached to the α-cytomembranes. Secondly, small vesicles, 300-1,000 A in width, with single smooth membranes, are found in all parts of the cell. When encountered close to the cell surface, the vesicles are sometimes arranged in rows (Fig. 13) similar to acetazolamide-treated material. A third component is the homogenous matrix in which all cell components are embedded.

2. *Pigment Epithelium.*—*a.* Cell Membrane and β-Cytomembranes: The β-cytomembranes are encountered close to the cell surface, that is, in that part of the cells which faces the external limiting membrane (Figs. 14 and 15). In general appearance, composition, and size, they are identical with the β-cytomembranes of the nonpigmented epithelium. They seem, however, to be less frequent, and they do not reach as deep into the cell body. This means that the β-cytomembranes in the pigment cells are found within a very small area next to the cell surface. No differences have been noted among rabbit, monkey, and human eyes as to the β-cytomembranes of the pigment epithelium.

Digit-like extensions from one cell to another also occur in the pigment layer, so that the boundary line between two adjacent cells is a very folded double membrane, similar to the nonpigmented epithelium (Fig. 16). The differences are that in the pigmented layer the extensions are present at the basal parts of the cells, are smaller, and are less frequent.

b. α-Cytomembranes: α-cytomembranes are also seen in the pigment cells. They are scattered in the cytoplasm of the cell and do not differ in any respect from the α-cytomembranes in the nonpigmented cells. Nor are there differences in this respect among the species examined.

c. Golgi Apparatus: In the pigmented cell, the Golgi apparatus is constantly located in the apical region and occupies a very small area (Figs. 8 and 18). In general appearance and composition it is similar to the Golgi apparatus in the nonpigmented cells, but the minute structure differs in some respect. In the pigmented cells the membranes are fewer and considerably shorter, and the vesicles found within the Golgi region are much less frequent. Thus the Golgi apparatus is limited to a very restricted area of the pigmented cells and is seen in relatively few sections. The differences in appearance of the Golgi apparatus between the pigmented and nonpigmented cells are similar in the three species.

d. Mitochondria: The ultrastructure of a mitochondrion of pigmented epithelium resembles that of the nonpigmented cell. However, there are fewer mitochondria in the pigmented cell, and they appear unevenly distributed in pigmented animals. In the cells where pigment granules are present, mitochondria are occasionally found between the granules, but they are mostly concentrated within the basal part of the cell, where pigment granules are absent. In albino animals, however, the mitochondria are scattered all over the cell without any particular concentration.

In the rabbit eyes the width of the mitochondria in the pigmented cells is the same as in the nonpigmented cell (0.17μ). In the two other species they are considerably smaller. In the monkey eyes the width is only 0.17μ and in the human eye it is 0.18μ as compared with 0.26μ and 0.22μ respectively, for the nonpigmented epithelium of these species.

e. Nucleus: The nucleus of the pigment epithelium in all three species occupies a comparatively large area in the cell and has a pronounced oval shape with its long axis parallel to the long axis of the cell.

f. Granules: There are two types of large granules in the pigment epithelium, pigmented and nonpigmented (Fig. 17). Although one can find individual granules with some pigment in albino animals, the majority of the granules in these animals are nonpigmented.

The pigment granules are located within the apical two-thirds of the cell, where they are distributed around the nucleus in the form of a cap. Only occasionally one finds granules in the most basal part of the cell.

There seem to be at least three different kinds of pigment granules. The commonest form is spherical with a diameter of 0.5μ-1.2μ in rabbit and monkey and 0.4μ-1.7μ in human eyes. Less common is the egg-shaped granule with sizes in the range of $1.0\times0.6\mu$-$1.4\times0.8\mu$ in rabbit and monkey and $1.0\times0.4\mu$-$2.5\times0.9\mu$ in human material. Rarest is the giant granule with a diameter up to 2.7μ. So far the last type has been seen only in the rabbit. Although each granule is very sharply limited from the surrounding, it has not been possible to visualize a definite limiting membrane. However, such a membrane may be masked entirely by the pigment.

In rabbit and monkey each granule appears separately, but in man one occasionally sees conglomerates of 12-15 relatively small granules. Each granule in all three species is built up of subunits, 0.05μ-0.1μ in size. The amount of pigment varies considerably from very heavily pigmented granules, where the subunits hardly can be identified, to granules with only a few subunits embedded in a homogenous ground substance (Fig. 17).

Nonpigmented (Fig. 18) granules are commonest in albino rabbits but occur to less degree in pigmented rabbits, monkeys, and man. In human eyes they have been found in only a few sections. These particular granules usually have an oval shape and a relatively uniform size, varying from 0.3μ to 0.5μ. They are without exception limited by a single membrane. Although they are called "nonpigmented," it is common to find such granules with several

dark grains scattered in the ground substance, even in albino rabbits.

g. Ground Substance: The ground substance of the pigment epithelium contains the same components as the nonpigmented epithelium. The same regular arrangement of the vesicles in rows is also observed in the pigment cells. However, in all three adult species, the ground substance of the pigment cells is much denser. This results in part from an increased number of the small granules, 150 A in size, scattered throughout the ground substance (Figs. 8 and 18).

h. External Limiting Membrane: On page 953 in this issue, the external limiting membranes of rabbit and man have been described. The differences in appearance of this membrane in the two species was pointed out. In the rabbit the external limiting membrane shows up as a very thin layer, 200-350 A thick; in the monkey it has a thickness of between 400 and 800 A, whereas, in man it occupies an area of up to 4μ in thickness (Figs. 14 and 19). In all three species the membrane is very homogenous and does not display any particular structure. The surface of the cell which faces the external limiting membrane is in all species very irregular with many cytoplasmatic extensions. In the rabbit and monkey the external limiting membrane follows the cell surface very closely, while in man the extensions lie embedded in the membrane (Fig. 19).

E. Alterations in Structure and Function

1. *Appearance of Epithelium in Young Rabbits.*—There is evidence that the newborn rabbit does not secrete aqueous humor. Kinsey et al. (1945) demonstrated that in rabbits up to 9 days of age the concentration of ascorbic acid in the aqueous is the same as in blood. Aqueous ascorbate reaches its normal level in the 27-day-old rabbit. Moreover, the same authors found the volume of the aqueous humor to be approximately zero in newborn rabbits. Therefore it might be of some interest to analyze the ultrastructure of the ciliary epithelium during the periods before and after the secretion of aqueous humor has started.

The material reviewed for this report includes rabbits 1 day, 5 days, and 30 days old.

Nonpigmented Epithelium: β-Cytomembranes. Although a large number of cells have been examined, β-cytomembranes have been found only in very few cells from 1- and 5-day-old rabbits. Not only are they almost absent in animals of this age group, but the membranes observed are very short. In 30-day-old animals however the β-cytomembranes are present and appear normal.

α-Cytomembranes. The number of α-cytomembranes is definitely increased in the young rabbits. They are found in all parts of the cell, and they do not appear in the groups seen in adult rabbits.

Golgi apparatus. The Golgi apparatus is found in the normal position, that is, in the basal part of the cells. The number of vesicles found in the periphery of the membrane system seems to be increased (Fig. 20) and the membranes are shorter.

Nucleus. It has been constantly observed in 1- and 5-day-old rabbits that the nucleus is located in the apical part of the cells. In many instances it is encountered immediately beneath the cell membrane (Fig. 21). In 30-day-old animals the nucleus has assumed its normal position in the basal part of the cell (Fig. 22).

Mitochondria. There is no appreciable difference in the appearance of the young rabbit mitochondria in comparison with the adult animal. There seems to be no difference in number of membranes, and the mean thickness is the same, 0.18μ.

Ground substance. The nonpigmented epithelium in the youngest rabbits (1 and 5 days old) has almost the same density as the pigment epithelium (Fig. 21). This is due to an enormous increase in the number of small electron-dense particles of the ground substance. In 30-day-old rabbits, on the other hand, there exists the same differ-

ence in density between the two epithelial layers as is observed in adult rabbits.

The small vesicles, encountered in the adult animals, are also present in the young ones. In young rabbits one may see the vesicles arranged in rows (Fig. 23), similar to normal and acetazolamide-treated adult animals (Fig. 13). Most interesting is the finding that this particular arrangement of the vesicles is also observed in rabbits (1-5 days old) with few, if any β-cytomembranes.

Pigment Epithelium: All cell components found in the pigment cells of adult animals are also present in the young rabbits. Thus, β-cytomembranes in a normal number are observed in one-day-old rabbits. This is surprising in view of the fact that they are practically absent in the nonpigmented epithelium at this age. There are also vesicles, and, as in adult animals, they are sometimes arranged in rows at the basal surface of the cell.

2. *Appearance of Epithelium After Treatment with Acetazolamide.*—The morphological changes seen after acetazolamide seem to be limited to the nonpigmented epithelium in rabbits and to the pigment epithelium in man. In both species the same cellular components are altered, namely, the mitochondria, the Golgi apparatus, and the ground substance of the cytoplasm.

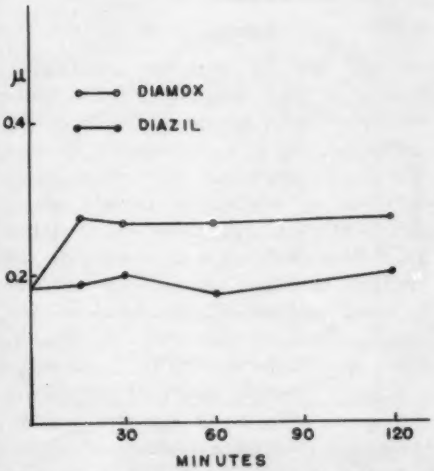

Chart 1.—Mean width of the mitochondria after intravenous administration of acetazolamide (Diamox) and Diazil (10 mg. per kilogram) to the rabbit. Reproduced from Holmberg, A.: Thesis, Stockholm, 1957.

Mitochondria: In rabbits treated with acetazolamide there is a marked increase in thickness of the mitochondria of the nonpigmented epithelium (Chart 1). The mean width of the mitochondria in normal and control rabbits is 0.18μ and in the acetazolamide-treated animals, 0.25μ. The difference is statistically significant. In the one human eye studied after acetazolamide the width of the mitochondria in the pigment epithelium was 0.20μ as compared to 0.18μ in two normal human eyes. The change seen in both species seems to be due to an increase of the ground substance of the mitochondria. No alterations of their fine structure have been observed. The mitochondria in the rabbit pigment epithelium and the mitochondria in the human nonpigmented epithelium are not influenced by acetazolamide, neither as to thickness nor fine structure.

Golgi Apparatus: The changes in Golgi apparatus after treatment with acetazolamide are shown in Figures 24 and 25 and Chart 2. Parallel with a decrease in the length of the membranes of the Golgi apparatus there is a marked increase in the number of small vesicles. Both species show the same alterations, and again in rabbits it is only the Golgi apparatus of the nonpigmented epithelium and in man the Golgi apparatus of the pigment epithelium which are affected by acetazolamide. The minute structures of the Golgi apparatus, such as the thickness of and the distances between the membranes and the dimensions of the vesicles, do not show any significant alteration after treatment with acetazolamide. Contrary to the changes in the mitochondria, which persist in all material treated with acetazolamide, the changes in the Golgi apparatus are most pronounced in material treated for a short period (15-30 minutes). In animals treated for an hour or more there are little or no changes in the Golgi apparatus as compared with control material.

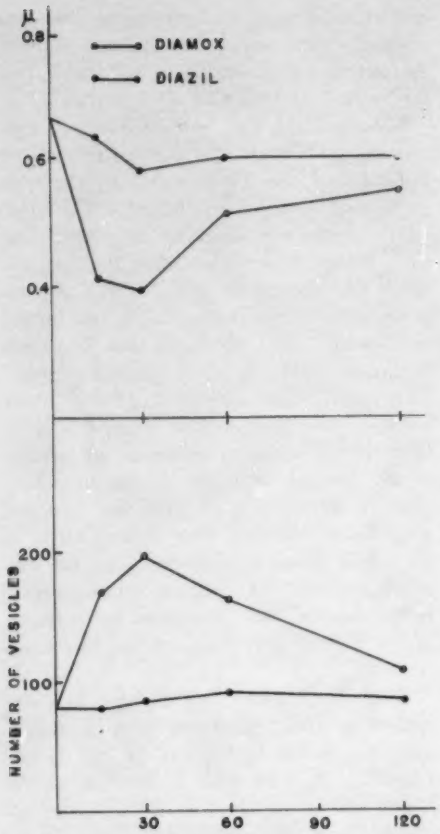

Chart 2.—Changes in the length of the membranes (upper diagram) and the number of vesicles (lower diagram) in the Golgi region after intravenous administration of acetazolamide (Diamox) and Diazil to the rabbit. Reproduced from Holmberg, A.: Thesis, Stockholm, 1957.

Ground Substance of Cytoplasm: As seen in Figures 25-29, in acetazolamide-treated rabbit and human eyes there is a marked increase in small vesicles in the cytoplasm as compared with the untreated material. Regarding the appearance of these vesicles, there seems to be some difference between the two species. In the nonpigmented epithelium of rabbits only one kind of vesicle is found, and this is the same as is seen in normal and control material. They are 300-1,000 A in size and have a smooth single membrane. In the pigment epithelium of man, two types of vesicles appear after acetazolamide. One is similar to that found in rabbits. The other type, and this is the majority, is larger and rather irregular in shape. It is limited by a single membrane, which in many instances has small dark granules attached to it. This suggests that these particular vesicles are derived from the α-cytomembranes.

In the rabbit, the number of vesicles decreases with time after treatment with acetazolamide (Chart 3). By one hour after the injection of acetazolamide, the frequency of vesicles is almost the same as in the controls. As only one patient has been studied after acetazolamide so far, there is no data of the changes in frequency of vesicles in human material.

A striking feature about the vesicles in rabbits is the fact that close to the cell surface of the nonpigmented cells they are arranged in parallel rows or concentric circles (Figs. 30-32). As has been mentioned above, this particular arrangement of the vesicles has been observed occasionally in normal material. The acetazola-

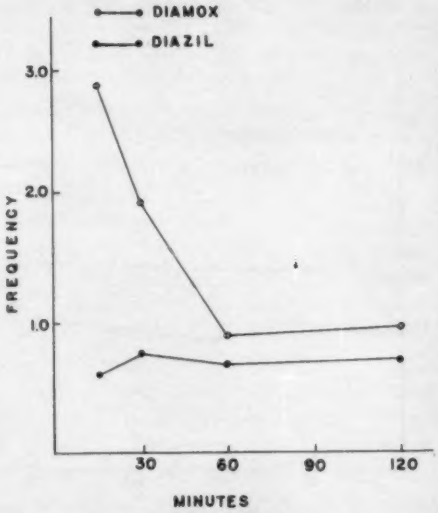

Chart 3.—Frequency of vesicles in the cytoplasm of the nonpigmented epithelium after intravenous administration of acetazolamide (Diamox) and Diazil to the rabbit. Reproduced from Holmberg, A.: Thesis, Stockholm, 1957.

mide-treated animals however, show this regular arrangement much more frequently. Futhermore, it can be seen even in animals treated for longer periods (two hours) in which the frequency of vesicles elsewhere in the cells is about normal.

The regular arrangement of the vesicles close to the cell surface suggests that there may exist some relationship between the vesicles and the β-cytomembranes, a fact which will be discussed later. It should be mentioned, however, that the number of β-cytomembranes is roughly the same in acetazolamide-treated animals as in normals and controls.

In the human material treated with acetazolamide no particular arrangement of the vesicles has been observed. They are encountered all over the cell in equal number, and the β-cytomembranes seem to be as frequent as in normal material.

Finally it must be reemphasized that all changes described after treatment with acetazolamide are limited entirely to the nonpigmented epithelium in rabbits and entirely to the pigment epithelium in man. No changes whatsoever have been observed in the pigment epithelium of rabbits or the nonpigmented epithelium of man.

F. Comment

Physiologic concepts of the aqueous humor and its formation have considered the tissue separating the blood from the posterior chamber as a single membrane. Unfortunately the anatomical basis for aqueous humor production is not that simple. As has been shown in this investigation, the ciliary processes have a complex structure with several layers of different kinds of tissue. Besides the two layers of epithelial cells there exist two limiting membranes which, at least in human eyes, reach considerable thickness. Further, there is a capillary wall with a basement membrane, and finally there is a layer of connective tissue.

β-Cytomembranes.—Most characteristic features of the ciliary epithelium are the β-cytomembranes (Holmberg, 1955, 1956, 1957; Pease, 1956; Pappas and Smelser, 1958). It is interesting to note that β-cytomembranes are present both at the place where fluid is supposed to enter a cell, that is, at the basal part of the pigment epithelium, as well as at the site where fluid leaves the cell, that is, at the apical part of the nonpigmented epithelium. It is further interesting to observe that at both places there is a membrane or a layer of material outside the cell membrane which the fluid has to pass before it can reach the cell (pigment epithelium) or leave the ciliary body (nonpigmented epithelium).

β-Cytomembranes are not unique for the ciliary epithelium, but are found in several other cells, such as the renal tubular cells (Rhodin, 1954), choroid plexus (Pease, 1956), some salivary glands (Pease, 1956), thyroid gland (Ekholm and Sjöstrand, 1957), and the pigment epithelium of the retina (Garron, unpublished). All these cells have in common the transport of fluid and ions in one or the other direction in relation to the blood. It is therefore believed β-cytomembranes are involved in the mechanism of this transport. Further support for this assumption is the fact that in young rabbits, in which secretion of aqueous humor has not yet started, one finds no membranes in the nonpigmented epithelium.

The possible role of the β-cytomembranes in the process of aqueous humor formation is a matter of speculation. They provide a mechanism by which the cell surface is increased. The β-cytomembranes in both epithelial cells may also be a site in which is localized enzymatic activity necessary for transport of substances across the cell membrane. This assumption is based on the fact that the β-cytomembranes are highly organized structures, resembling the mitochondria with their known high enzymatic activity.

The β-cytomembranes can be formed in two ways. They can be formed either by downgrowth of the cell membrane or by fusion of the small vesicles which are

present in the cytoplasm. From a static picture it is impossible to decide. The fact, however, that in young rabbits one hardly finds any β-cytomembranes in the nonpigmented epithelium but instead groups of vesicles arranged in rows close to the cell surface suggests that the β-cytomembranes are formed by fusion of these vesicles.

In connection with the β-cytomembranes it might be pertinent to discuss some of the findings after treatment with acetazolamide. Pappas and Smelser (1958) explain the accumulation of the vesicles, and especially those which are regularly arranged in rows beneath the cell membrane, as a result of a breakdown of the β-cytomembranes. Several facts do not support this explanation, however. If one assumes that the β-cytomembranes have similar function in both epithelia, it is difficult to understand why the membranes in the pigment epithelium are not affected by acetazolamide. Futhermore, it is not uncommon to observe vesicles in the same arrangement in normal eyes in the presence of β-cytomembranes. In young rabbits one only finds vesicles and rarely membranes. Finally, a rough estimation of the number of β-cytomembranes in acetazolamide-treated rabbits does not show any significant difference as compared with the controls or the normal animals. Instead of being a sign of a breakdown of the β-cytomembranes, the presence of vesicles in a regular arrangement at the cell surface may be a sign of the formation of the β-cytomembranes by confluence of the vesicles.

α-Cytomembranes.—The cell component named α-cytomembranes (Sjöstrand, 1956) is identical with the endoplasmic reticulum and ergastoplasm.

This particular structure appears significant for the cells of some excretory glands, such as pancreas and the salivary glands. The α-cytomembranes in these cells may be involved in the synthesis of the proteins in the secretory product.

There is no abundance of α-cytomembranes in the ciliary epithelium, although there is a difference in the number between the two layers. As no protein is present in the aqueous humor, their role may be limited to the synthesis of protein for the cell itself. From this point of view it is interesting to find a high amount of α-cytomembranes in young animals in both layers. The fact, however, that in human eyes the α-cytomembranes in the pigment epithelium seem to be influenced by acetazolamide may suggest that they are involved in some way in the formation of aqueous humor.

In human material, the vesicles, seen in the pigment epithelium after acetazolamide treatment, often have granules attached to their membranes. Therefore, they are probably derived from the α-cytomembranes. In the rabbit nonpigmented epithelium, however, where an enormous accumulation of vesicles occur after the same treatment, all vesicles have a smooth membrane. Granules have not been observed attached to the membrane surrounding the rabbit vesicles. Therefore, there are no morphological proofs that these vesicles are derived from the α-cytomembranes. Furthermore, the number of α-cytomembranes seems to be the same in acetazolamide-treated animals as in control and normal animals.

Golgi Apparatus.—In composition the Golgi apparatus in both ciliary epithelia has a very close resemblance to the Golgi apparatus in the proximal tubular cells of the kidney (Rhodin, 1954). It differs, however, from that of the excretory cells in the pancreas, where the small units, seen around the membrane system, are granules instead of vesicles (Sjöstrand and Hanzon, 1954). Because of the close relation to the membranes it has been assumed that the small units which appear as granules or vesicles are derived from the membranes. Evidence for this assumption may be derived from the effect of acetazolamide, which causes a decrease of the length of the membranes at the same time as it increases the number of vesicles.

The Golgi apparatus is believed to be connected to the secretory process of the

cell. This is supported by the fact that acetazolamide alters the morphology of the Golgi apparatus. Another interesting finding from the same point of view is the location of the Golgi apparatus in the two layers in relation to each other. As has been described, they lie very close together. Topographically this could be explained from an embryological standpoint, the Golgi apparatus actually being located in both layers in the apical part of the cell.

Mitochondria.—In all tissues investigated with electron microscopy so far, the mitochondria are principly built in the same way. They are always surrounded by a triple-layered membrane, and in the homogenous matrix one finds several triple-layered parallel membranes. It is reasonable to believe that this particular arrangement provides some anatomical basis for the enzymatic activity.

In the ciliary epithelium it is interesting to note that after treatment with acetazolamide there is an increase in the thickness of the mitochondria. This observation, however, is limited to that cell layer where other changes are seen after the same treatment, that is, the rabbit nonpigmented epithelium and the human pigment epithelium.

Granules.—Different types of pigment granules in the human ciliary epithelium have been described earlier (spherical, egg-shaped, and giant granules) by Binder and Orth (1953). Sebruyns (1951) found that the individual granule in the human retina was composed of very small units. These observations were made on isolated granules in both cases. These findings have been confirmed in observations on sectioned material. Contrary to Sebruyns, however, no fibrils attached to the granules have been seen.

It is of interest that in albino animals one finds in the pigment epithelium homogenous granules, which in some instances contains dark grains. It seems reasonable to consider these granules as equivalent to the pigment granules in the pigmented animals. In support of this assumption is the fact that in pigmented animals one can follow series of granules from a stage where no pigment is present through stages with an increasing amount of pigment to the completely developed granule.

Ground Substance.—In the ground substance of both normal epithelia of all three species there are a certain number of small vesicles. They are encountered in all parts of the cell and are identical morphologically with the vesicles seen in the Golgi apparatus.

The regular arrangement of the vesicles, which is observed near the cell surface in both normal and acetazolamide-treated material is striking and suggestive of a relationship between the vesicles and the β-cytomembranes. The same phenomenon occurs in the tubular cells of the kidney but has been interpreted as perforations of the β-cytomembranes (Bargmann, et al., 1955; Berkaloff, 1959). This is not the case in the ciliary epithelium as is evident from analysis of serial sections. In the kidney it has also been interpreted as a variation in the function of the cell (Berkaloff, 1959), and this is of interest in view of the effect of acetazolamide upon the ciliary epithelium.

From a static picture it is impossible to determine the exact relationship between the vesicles and the β-cytomembranes. However, as has been mentioned previously, there are several evidences which favor the assumption that the vesicles form the β-cytomembranes by fusion.

The remarkable accumulation of the vesicles present in normal material after early stages of acetazolamide treatment is correlated in both species to the period when the intraocular tension is falling. When the tension is stabilized on a low level and when one can assume that the secretion of aqueous humor has reached a new steady state, the frequency of vesicles has diminished to almost normal. Further, after acetazolamide treatment one more often sees vesicles arranged in rows near the cell surface. All these observations suggest that

the vesicles are involved in the secretory process.

Finally, it should be noted that the control animals showed no changes of ultrastructure as compared with the normal.

G. Conclusion

A detailed analysis of the ciliary epithelium has revealed a complex structure, which is essentially the same in all three species examined. As similar structures have been observed in other cells, where a transport of fluid and ions takes place across the cell, one may conclude that they are of importance for this process. From the same standpoint it is of interest to find exactly the same structure, where fluid enters and where it leaves the epithelium.

Acetazolamide (Diamox) affects the morphology remarkably, but when interpreting the findings in relation to the functional changes one must consider possible nonspecific effects. It is conceivable that lowering of intraocular tension in itself would produce the changes observed. In that case, however, one would expect to find the same alterations in both epithelia and one would expect the changes to remain as long as the tension is kept on a low level. Finally, in an attempt to inhibit the secretion of aqueous humor by increasing the intraocular tension, Pappas and Smelser (1958) obtained exactly the same morphological picture as after acetazolamide treatment. Thus it seems highly unlikely that variation in tension would have any influence on the morphology.

Another fact to consider and probably the most difficult one to control is pH in the cell. An alteration of pH can possibly account for all changes seen. To my knowledge, there are no reports about the effect of pH on the ultrastructure in the living cell. However, there are experiments done (Rhodin, 1954; Zetterqvist, 1956) which show that a variation of pH of the fixation solution within wide ranges does not alter the ultrastructure to any appreciable degree. It must also be emphasized that the changes in the mitochondrial width persist through all experiments, whereas the number of vesicles in the cytoplasm and Golgi apparatus decreases very rapidly when the time of acetazolamide treatment increases. Therefore, one is justified in assuming that pH *per se* does not have any influence of importance upon the morphological picture.

From the discussion above it is reasonable to conclude that the decrease of secretion of aqueous humor, induced by acetazolamide, is a result of a change of the anatomical structure.

The most impressive finding in the acetazolamide experiments is the enormous increase in the number of small vesicles, present in normal material. This observation is made only in the early stages of acetazolamide treatment, which means it is related to the time when the inhibition of the secretion has just started. The close relationship between the accumulation of vesicles and the decrease in aqueous output suggests that the vesicles carry aqueous. This leads to the conclusion that the process involved in the secretion of aqueous humor would also include a phenomenon called pinocytosis. This phenomenon is known to occur in living cells (Lewis, 1933), although it has never been thought of as a mechanism in secretory activity. However, from a static picture it is impossible to decide whether or in what direction the vesicles are moving.

The assumption that the vesicles carry aqueous to the cell surface also means that aqueous is formed into the vesicles across the membrane which surrounds them. Therefore one can consider the formation of aqueous humor as an intracellular process and not as a process which only takes place at the border between the epithelium and the posterior chamber, that is, across the cell membrane. In other words, the aqueous humor is formed before it reaches the cell surface.

The effect of acetazolamide on human ciliary epithelium as compared with the effect in rabbits is intriguing, but one needs

more data before any conclusions can be drawn. Although belonging to different cell layers, it is of interest, however, that exactly the same cell components are involved in both species. The difference of the effect of acetazolamide on rabbit and human ciliary epithelium may be due to the difference in composition between rabbit and human aqueous humor. It may also suggest that the pigment epithelium plays a more direct role in the formation of aqueous humor than earlier was assumed.

H. Summary

The fine structures of the ciliary epithelia of rabbit, monkey, and human eyes are described. The different cell components are discussed in comparison to other cells with similar function. It is found that characteristic of all these cells is an abundance of β-cytomembranes.

In an attempt to correlate the morphological picture with the functional activity of the ciliary epithelium, experiments have been performed with inhibition of aqueous humor secretion by acetazolamide (Diamox). These experiments were done in adult nephrectomized rabbits and in one human eye. Further, the structure of the ciliary epithelium has been studied in young rabbits, where the secretion of aqueous humor has not yet started or has just started. The findings obtained in all material suggest that at least part of the aqueous humor is primarily formed within the cells into small vesicles which transport the aqueous humor to the cell surface.

From the results obtained it is possible that the human pigment epithelium plays a more important role in the production of aqueous humor than does the rabbit pigment epithelium. However, it is necessary to examine human eyes at longer time intervals after acetazolamide treatment before definite conclusions can be drawn.

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The Ultrastructure of the Capillaries in the Ciliary Body

AKE HOLMBERG, M.D., St. Louis

Introduction

During the analysis of the fine structure of the ciliary epithelium (Holmberg, 1957; Holmberg, 1959) in human and rabbit eyes, it was observed that the capillaries in the stroma are built up in a characteristic way, similar to that found in several secretory glands. Although the findings are not unique for the ciliary body, they add to our knowledge of the correlation between structure and function of the ciliary processes.

Material and Methods

In this investigation the same rabbit material has been utilized as was used for the analysis of the nonpigmented epithelium. All fixation was performed in vivo in 1% osmium solution with the animals under general anesthesia (pentobarbital [Nembutal] intravenously). After 10-15 minutes parts of the ciliary body were cut out and fixation was continued for 3-4 hours.

The human material consists of three fresh eyes enucleated because of malignant melanoma in the posterior pole. Immediately after the enucleation 1% osmium solution (Palade, 1952; Sjöstrand, 1953) was injected into the posterior chamber. After about 10-15 minutes parts of the ciliary body were removed for continued fixation for 2-3 hours. Both materials were dehydrated in series of ethyl alcohols and embedded in methacrylate. Sectioning was done partly with a Sjöstrand Ultramicrotome, and partly with a Porter-Blum microtome using glass knives. The sections were examined in an RCA Model EMU 2E microscope.

Results

The capillaries of the ciliary processes lie very close to the pigment epithelium. In rabbits a thin layer of connective tissue separates the capillary wall from the epithelium (Fig. 1). In the human eye there is in addition to the connective tissue layer, a thick external limiting membrane (Fig. 2), as has been described on page 953 in this issue.

In both species the capillary wall consists mostly of a single endothelial layer, well defined on both sides by a cell membrane (Fig. 3). Where two adjacent cells meet there is usually an overlapping, so the wall within these areas is made up of two thin endothelial layers, separated from each other by a very regular, narrow space (Fig. 4). In other places two adjacent cells meet end to end with the cell membranes in close contact with each other (Fig. 3).

The capillary walls are extremely thin in most parts. The thickness of the wall is about 0.15μ, but in many regions it is thinned out to a layer not more than about 150 A. wide. Within both areas one finds only a homogenous cytoplasmic ground substance in which are scattered small vesicles and occasionally a mitochondrion (Fig. 3). It is only where the nucleus is

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From the Departments of Ophthalmology, and Anatomy, and the Oscar Johnson Institute, Washington University School of Medicine.

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situated that the capillary wall becomes wider. Even at these places it is relatively thin, and the nucleus is elongated and flat (Fig. 5). Around the nucleus one finds a greater amount of cytoplasm with the usual cell components, such as mitochondria, α-cytomembranes, Golgi apparatus, and small vesicles (Fig. 5). On the outside surface, that is on the surface towards the ciliary stroma, the capillary wall is covered by a basement membrane (Fig. 3), which shows the usual characteristics for such membranes. Thus it has a comparatively low density, and it is ill-defined. The thickness is about 300 A. and it is separated in most places from the endothelial cell membrane by a thin light space, about 150 A. wide.

From a functional point of view, the most important finding in the ciliary capillary wall is the presence of discontinuities or pores in the wall (Figs. 6 and 7). They exist in both human and rabbit material and are of the same size in both species. The size varies from 200 A. to 1200 A. The basement membrane on the outside surface of the wall continues uninterrupted over the pores. Rarely, it has been observed that the pores are bridged over by a thin single membrane (Fig. 6). As compared to the capillaries of some other tissues, the pores in the capillaries concerned in this investigation, seem to have a relatively low frequency. Thus in 150 different sections through the capillary wall of 16 rabbit and 3 human eyes, only 25 pores have been seen.

Comment

The ultrastructure of the capillary wall in the ciliary processes is by no means unique. In some secretory glands such as thyroid (Monroe, 1952; Ekholm, 1957), adrenal cortex (Zelander, 1957), and parathyroid (Trier, 1958), the capillary wall is extremely thin with discontinuities about 400 A. wide. Thus, these display much the same characteristics as the ciliary body capillaries, although in the latter the pores seem to be less frequent. Concerning capillaries, the most excellent example of a highly specialized ultrastructure is found in the kidney glomeruli (Rhodin, 1955; Moore et al., 1957). Here the capillary wall is very markedly and regularly fenestrated by pores with an average size of 0.05μ-0.1μ (Rhodin, 1955). Differing from the capillaries mentioned so far are those in the lung (Bargmann et al., 1956, Schultz et al., 1957; Woodside et al., 1958) and the pituitary (Bargmann et al., 1957), which have no pores, even though their walls are very thin. The capillary walls in heart and skeleton muscles also lack pores (Moore et al., 1958).

The significance of a fenestrated capillary wall has been demonstrated best in the glomeruli. By examining different species, Hall (1957) has shown that the filtration rate through the glomeruli correlates with the grade of fenestration of the capillary wall. Thus, for instance, the filtration rate is about 100 times higher in the glomeruli, as compared with muscles. Therefore it seems reasonable to believe that the pores account for the fluid transport through the capillary wall. The small vesicles seen in the cytoplasm are probably without much importance in this respect, at least in capillaries provided with pores.

The presence of pores in the ciliary capillaries suggests that the capillary wall does not constitute a barrier for fluid transport. It also indicates that in the ciliary processes there is likely a comparatively high filtration from the blood to the ciliary stroma and epithelium.

Summary

The ultrastructure of the capillary walls in the ciliary processes of human and rabbit eyes is described. As in some other tissue capillaries, the wall is extremely thin and provided with pores, 200 A. to 1200 A. wide. Both species examined display the same features. The role of the pores in relation to fluid exchange between blood and the surroundings is discussed, and it is suggested that the capillary wall in the

ciliary processes probably does not form a barrier between blood and aqueous.

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Differences in Ultrastructure of Normal Human and Rabbit Ciliary Epithelium

AKE HOLMBERG, M.D., St. Louis

Introduction

In a previous investigation (Holmberg, 1957), an extensive analysis of the ultrastructure of the nonpigmented epithelium in rabbit eyes was carried out. The material included both the normal structure and changes in the structure after inhibition of the secretion of aqueous humor by acetazolamide (Diamox). In order to find out the effect of acetazolamide on the structure of human ciliary epithelium, an analysis of the normal ultrastructure of this epithelium has been started. As there proved to be remarkable differences as compared to the rabbit material, a preliminary report of these differences seems to be justified.

Material and Methods

So far three enucleated human eyes have been examined. The eyes were enucleated because of a malignant melanoma in the posterior pole. No clinical signs of altered secretion of aqueous humor could be detected. The operations were performed under local anesthesia (retrobulbar injection of Xylocaine). Immediately after the enucleation 1% osmium tetroxide solution (Palade, 1952; Sjöstrand, 1953) was injected into the posterior chamber. After fixation in situ for about 15 minutes, parts of the ciliary body were removed and fixed for another 2-3 hours. Embedding was done in methacrylate according to Newman and Borysko (1949). Sectioning was performed on a Porter-Blum microtome using glass knives. The sections were examined in an RCA Model EMU 2E electron microscope.

Results

The most striking differences in the ultrastructure between human and rabbit ciliary epithelium are first the appearance and extension of the internal and external limiting membranes, and second the structure and frequency of granules in the nonpigmented epithelium.

The Internal Limiting Membrane.—In rabbits the internal limiting membrane consists of a rather ill-defined membrane of comparatively low contrast (Fig. 1). The thickness of this membrane varies between 290 A. and 370 A. (Holmberg, 1957) and it is separated from the cell membrane by a light space, 400 A. to 700 A. wide.

The corresponding membrane in the human eye consists of a meshwork of anastomosing osmiophilic bands bordering areas of different sizes and shapes (Fig. 2). The meshwork occupies a region outside the cell membrane varying in thickness between 0.3μ to 3.6μ. The individual band in the meshwork has a rather low contrast and lacks a definite border line. Each band is between 250 A. to 500 A. wide and seems to be quite homogenous. No striation or periodicity has been observed. The meshes formed by the osmiophilic bands vary in size from 0.37μ to 1.1μ. The shape

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From the Departments of Ophthalmology, and Anatomy, and the Oscar Johnson Institute, Washington University School of Medicine.

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is mostly oval, but circular, triangular, or quite irregular meshes have been encountered. They appear to be electron optically empty, but occasionally meshes are observed filled with a homogenous, structureless material (Figs. 2 and 4).

As has been mentioned above, the thickness of the internal limiting membrane in human eyes varies considerably. In some limited areas it is reduced to a single membrane (Fig. 5) similar to the condition in the rabbit eye. However, the meshwork is constantly found within all the indentations between adjacent cells (Fig. 6). The posterior chamber side of the meshwork is outlined by a continuous membrane, 500 A. to 700 A. thick (Figs. 2 and 4). This membrane in itself is however somewhat diffuse and shows the same grade of contrast as the osmiophilic bands in the meshwork. There are frequent anastomosis between the membrane and the meshwork.

The External Limiting Membrane.—The appearance of the external limiting membrane in the rabbits is that of a usual basement membrane with its characteristics (Figs. 7 and 8). Thus, it is not sharply limited and the contrast is low. No structure has been observed in the membrane and it seems homogenous. The thickness varies between 190 A. and 300 A. In most places it is separated from the cell membrane of the pigmented cells by a light space, 120 A. to 360 A. wide. In some regions, however, there is no such space, and the external limiting membrane lies in close contact with the cell membrane. In general, the basal surface of the pigmented cells is smooth, but now and then longer or smaller cytoplasmic extensions are seen making the cell surface irregular. The external limiting membrane follows the irregularities very closely.

In human eyes the anatomical conditions are quite different. Instead of a thin basement membrane, the external limiting membrane occupies a tremendously large space, varying in thickness from 1.5μ to 4.0μ (Fig. 9). The layer is homogeneous and even in thin sections with highest possible resolution, it has been impossible to reveal any distinct structures. It always lies in close contact with the cell membrane of the pigmented epithelium. No light space has been observed as in the rabbit. The border to the ciliary stroma is sharp, but a special limiting membrane is not present. In some areas, all of the spaces between the pigmented cells and the capillaries in the stroma are occupied by this homogenous structureless material. In other instances, a thin layer of stroma is present between them (Fig. 9).

The basal surface of the pigmented epithelium is very irregular with a large number of cytoplasmic extensions, which reach deep down in the "basement membrane." Within this layer, large groups of cytoplasmic islands have been observed (Figs. 9 and 10).

Granules in the Nonpigmented Epithelium.—In a previous report (Holmberg, 1957), granules have been described in the nonpigmented epithelium in the rabbit. These granules are located for the most part toward the basal regions of the cells. They always appear singly and contain clusters of fine dark grains. In human material there are an abundance of granules, but they show a completely different structure (Fig. 11). Almost without exceptions, the granules are in aggregates. In each section through a cell 10 to 15 such aggregates have been counted with up to 10 granules in each aggregate. In most cases the individual granule has an oval form with the size varying from 0.7μ by 0.03μ to 0.65μ by 0.43μ. They do not show any specific inner structure, but appear completely homogenous with pronounced osmiophilia. Occasionally, vacuolization of the granules has been observed with one or more central vacuoles.

Comment

In light microscopy, the limiting membranes are described as a continuous membrane covering the ciliary epithelium in all

its extensions (Saltzmann, 1912). Due to the comparatively limited resolving power of the light microscopy, it seems obvious that the cell membranes are included in the names internal and external limiting membranes. As has been shown in this investigation, however, it is clear that these membranes are definite entities, easily distinguished from the cell membranes.

On the whole both limiting membranes in the rabbits demonstrate the same characteristics. They are of the same size, have the same grade of contrast, and lack special structure. That is, they have the same appearance as a regular basement membrane. It is remarkable that in human material the same membranes not only differ in thickness and structure when compared with the rabbit, but also that they are completely different from each other.

The presence of membranes lining both surfaces of the epithelium is explained from an embryologic point of view, but the compositions of the membranes seem to be unique for the ciliary body, particularly human ciliary body.

What is seen as a meshwork in osmium fixed and methacrylate embedded material is with great probability only a part of the true composition of the internal limiting membrane in the living eye. The outlined areas with a homogenous material in the meshes suggest that some in vivo substances may have been dissolved during the fixation and dehydration.

With special staining technics, it has been suggested (Graumann and Rohen, 1958; Cibis and Yamashita, in preparation; Zimmerman, personal communication) that the internal limiting membrane in the human eye consists of mucopolysaccharides or similarly staining material. These may be dissolved completely or partly during the fixation procedure. It is possible that the homogenous material, which in the electron microscope is seen to occupy some meshes represents mucopolysaccharides. The osmiophilic bands in the meshwork on the other hand are probably proteins or material with a protein component and are most resistant to the fixation procedure.

The internal limiting membrane is interesting from several points of view. One of these is the relationship between the membrane and the zonules. Pappas and Smelser (1958) have reported that the zonules in rabbits are anchored in the internal limiting membrane.

Another interesting point for speculation is the possible role the membrane plays in the formation of aqueous humor. A membrane of the thickness and composition seen in human material might influence the transport of various substances passing through it.

While the external limiting membrane in the rabbit is a regular basement membrane, well known from other tissues, it is very surprising to find in the human eyes that the corresponding membrane reaches a thickness of several microns. Another point is that in human material there is no light space separating it from the cell membrane, as has been described for almost every tissue examined (Ottoson et al., 1953; Sjöstrand and Rhodin, 1953; Rhodin, 1954; Bargmann, 1955; Zetterqvist, 1956, and others). In rabbit ciliary epithelium the light space also does not always exist. These findings in human and rabbit material may indicate that the light space seen by so many observers actually is an artifact caused by shrinkage of the basement membrane during the fixation and dehydration. Hall (1957) has shown that the basement membranes in the glomeruli are in close contact with the cell membranes, and no light space (Rhodin, 1955 and others) exists in well fixed material.

The irregularity of the basal surface of the pigmented cells, most pronounced in human material, with long extensions deep into the external limiting membrane, increases the cell surface. This may be of great importance for the resorption of fluid by the cells.

With histochemical methods it has been shown (Graumann and Rohen, 1958; Cibis and Yamashita, in preparation) that the

external limiting membrane is periodic acid-Schiff (PAS) positive and presumably contains neutral mucopolysaccharides.

What role the external limiting membrane plays in the transportation of fluid from the blood capillaries to the epithelium is unknown.

Summary

Some remarkable differences in the ultrastructure between human and rabbit ciliary epithelium are described and discussed. The internal and external limiting membranes, which in rabbits show up as very thin membranes, appear as layers up to 4μ thick in human material.

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The Fine Structure of the Inner Wall of Schlemm's Canal

AKE HOLMBERG, M.D., St. Louis

Many aspects of the anatomy and histology of the normal trabecular meshwork are comparatively well known from light microscopy. Unsolved problems exist, however, such as the exact structure of the outermost layer of the meshwork and the existence of extracellular material in the intertrabecular spaces. This preliminary report considers these two problems, and indicates the potentialities of electron microscopy for assessing the detailed structure and topography of the trabecular meshwork.

Material and Methods

The material used in this investigation consists of three human and four monkey eyes. The human eyes were enucleated because of malignant melanoma of the posterior pole. In no cases were there any clinical signs of impaired outflow. All human material was fixed within 1-2 minutes after enucleation, and the monkey eyes were fixed in vivo before enucleation. The fixation procedure was the same, which is routinely used for electron microscopy. Sectioning was performed on a Porter-Blum microtome, and the sections were examined in an RCA Model EMU 2E electron microscope.

All the material presented in this report has been cut in the anterior-posterior direction.

Results

In general, the inner wall of Schlemm's canal is composed of two endothelial linings, one facing Schlemm's canal and one facing the intertrabecular spaces (Figs. 1 and 2). The two linings belong in most instances to different endothelial cells and only occasionally it has been observed that they are formed by one cell. Between the linings there is a narrow space, usually less than 1μ in width. In the anterior-posterior cutting direction used, it can be traced for long distances. In the space there is often encountered a homogenous material, the characteristics of which will be discussed below. An important finding is that the space is not closed, but has free communication with the underlying intertrabecular spaces (Fig. 2).

The endothelial cells which form the lining next to Schlemm's canal seem to be very specialized, and rather frequently display large spaces. On individual sections these appear as closed vacuoles (Figs. 1, 2, and 3), usually oval in shape, and with sizes in the range 0.5μ-1.0μ×1.0μ-3.0μ. However, a thorough analysis reveals that these "vacuoles" actually represent channels through the cells with communications both to the underlying space and to Schlemm's canal. Figure 4 is an individual section showing a large opening from the "vacuole" to the space underneath, which in turn has communication with the intertra-

The illustrations for this article will be found in special section of illustrations, pp. 1047 to 1056.

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Fight for Sight Fellow of the National Council to Combat Blindness.

From the Departments of Ophthalmology, and Anatomy, and the Oscar Johnson Institute, Washington University School of Medicine.

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becular spaces (Fig. 2). Figures 5-9 represent serial sections through several "vacuoles" definitely showing that there exist openings through the inner wall of Schlemm's canal. Not enough material has been collected yet to determine the frequency and size distribution of these openings or pores, but the size of those seen so far is of a magnitude of 0.5μ to 1.5μ. The endothelium forming the other lining and the endothelium covering the individual trabecula do not display any "vacuoles."

The other problem considered in this study was the presence of an extracellular material in the intertrabecular spaces. Very regularly one sees in the space nearest to Schlemm's canal a homogenous material (Fig. 2) which might be identical with that which stains metachromatically (Brini, 1956, Vrabec, 1957) and with Alcian blue or colloidal iron (Zimmerman, 1957, Cibis and Yamashita, in preparation).

If saccharated iron oxide is injected into the anterior chamber of monkey eyes and left there for about 15 minutes, one finds that the iron particles are localized exclusively within those areas where the homogenous material is present (Fig. 10). The iron has never been found intracellularly or in regions where no homogenous substance is visible. In other experiments hyaluronidase from beef testis (Wydase) in concentrations of 150 TRU to 600 TRU per ml. was perfused through the anterior chamber in monkey eyes for about one-half hour and followed by injection of iron. Unfortunately, the hyaluronidase used produced much distortion of the normal relationship between the different trabecular structures. This made it impossible to evaluate the effect of hyaluronidase upon the homogenous intertrabecular material.

Comment

From perfusion experiments using suspension of particles of different sizes (Huggert, 1955; Huggert et al., 1955; Karg et al., 1959) and from microradiographic studies (Francois et al., 1955), it appeared likely that there must exist direct communications between the anterior chamber and Schlemm's canal. Using data from entirely different kinds of experiments (Huggert et al., 1955; Bárány et al., 1953; Bárány, 1954; Bárány et al., 1955; Bárány, 1956, and Huggert, personal communication), Bárány has recently made a theoretical calculation, showing that the pathways should have a size between 0.6μ and 1.0μ.

Using regular light microscopy several investigators (Theobald, 1934, 1955; Ashton et al., 1956; Flocks, 1956; Unger, 1957; Rohen and Unger, 1957, and Speakman, 1959) have demonstrated that the intertrabecular spaces communicate with each other forming direct comparatively large pathways from the anterior chamber to the inner wall of Schlemm's canal. But analyzing this last part of the trabecular meshwork has met with great difficulties, due to the limited resolving power of the light microscope. Although some authors (Theobald, 1955; Ashton et al., 1956; Rohen and Unger, 1957, and Speakman, 1959) have demonstrated openings in the inner wall with sizes of the magnitude of 10μ, others (Flocks, 1956) believe that these are artifacts. The pathways are unlikely to be of that size according to Bárány's calculations. Furthermore, McEwen's (1958) calculations show that one single channel of 12μ is sufficient to account for the facility of carrying all the fluid from the anterior chamber to Schlemm's canal.

Even with the electron microscope, Garron et al. (1958) were unable to detect openings in the inner wall of Schlemm's canal, although they did not deny their existence.

By a thorough study of serial sections, however, it has been possible in this investigation to visualize the open pathways between the intertrabecular spaces and Schlemm's canal in both monkey and human eyes. It is of considerable interest that these pathways, contrary to those seen in light microscopy, are the size predicted by theoretical calculations.

The homogenous material found in the spaces beneath the inner wall of Schlemm's canal might very well be some form of mucopolysaccharide, such as that stained by Alcian blue or colloidal iron and removed by testicular hyaluronidase (Zimmerman, 1957; Cibis and Yamashita, in preparation). The fact that in the experiments with injection of colloidal iron in vivo the iron particles are found only within areas where this material is present may indicate that the material has an affinity for iron. However, before one concludes that it represents some form of mucopolysaccharide, it will be necessary to prove that the iron particles are not merely held in this material mechanically.

A detailed discussion of the ultrastructure of the trabecular meshwork will be presented in a forthcoming publication.

Summary

Open communications between the intertrabecular spaces and Schlemm's canal through the inner wall of Schlemm's canal have been demonstrated by electron microscopy. The pathways have a size in the range of 0.5μ-1.5μ.

In the intertrabecular spaces, one finds a homogenous material, which takes up colloidal iron, suggesting the possibility of its being some kind of acid mucopolysaccharide.

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Studies on the Ciliary Epithelium and the Zonule

II. Electron and Fluorescence Microscope Observations on the Function of Membrane Elaborations

GEORGE D. PAPPAS, Ph.D.; GEORGE K. SMELSER, Ph.D., and PHILIP W. BRANDT, M.S., New York

The two layers of cells forming the epithelium of the processes of the ciliary body are characterized by extensive elaborations of their membranes. The elaboration of the membranes of the cell layer facing the posterior chamber is of two types: (*a*) infoldings of the free surface facing the posterior chamber and (*b*) interdigitations of the margins between adjacent cells (Pappas and Smelser [1] and Holmberg [2]). Similar elaborations of cell membranes have been found in cells of other tissues engaged in secretion (Pease [3]).

It was reported previously that the experimental alteration of normal aqueous humor secretion by various agents produces changes in the fine structure of the cells. The most obvious changes noted were an increase in the number of vesicles in the cytoplasm and the concomitant disappearance of the infoldings and interdigitations.[1] Thus the loss of the infoldings and interdigitations of the ciliary epithelial cells is a morphologic change correlated with the disruption of normal aqueous humor secretion. The changes in the configuration of these membranes suggest to us that the elaborations are involved in secretory function.

In order to determine the role of the membrane elaborations in secretion and possible reabsorption of material from the posterior chamber, studies were carried out to trace the passage of materials from the blood through the ciliary epithelium into the posterior chamber.

Sodium fluoresceinate was introduced systemically, and its passage through the tissue, which was prepared by freeze-drying, was followed with the fluorescence microscope. The higher resolving power of the electron microscope was used to follow distribution of the electron-dense particles of thorium dioxide which were injected either intravenously or directly into the posterior chamber.

In addition, the normal development of the surface epithelial cells was studied in relation to the appearance of secretory activity. Following is a preliminary report of these observations on the structural basis of aqueous formation.

Materials and Methods

Ciliary processes from adult (2.5-3.0 kg.) and newborn to 14-day-old albino rabbits were studied. Prior to removal of the eye, the rabbits were lightly anesthetized with pentobarbital (Nembutal), and dibucaine (Nupercaine) was applied topically.

In order to fix the material for electron microscopy, a solution of 1% osmium tetroxide in 1/14 M barbital (Veronal)-acetate buffer, pH 8.1, was injected directly into the posterior chamber of the eye of the

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Departments of Anatomy and Ophthalmology, Columbia University College of Physicians and Surgeons.

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anesthetized animal. A keratome incision was made at the limbus opposite the injection site to relieve pressure during the injection. After removal of the eye, it was rapidly dissected in the fixative. Small pieces of the ciliary process were fixed 20 to 30 minutes. The tissue was subsequently dehydrated in alcohol and embedded in a mixture of 90% butyl and 10% methyl methacrylate and the blocks polymerized overnight at 45 C. Thin sections were cut on a Porter-Blum microtome, placed on carbon-coated grids, and examined with an RCA EMU 3C electron microscope.

In order to study the distribution of the water-soluble sodium fluoresceinate in the normal ciliary processes, 10 ml. of a 10% solution of this dye was injected intravenously. As soon as fluorescein appeared in the aqueous, the animal was anesthetized and the eye removed. Small pieces were cut from the ciliary process and frozen in isopentate at −140 C. The tissue was dried and embedded in vacuo in diethylene glycol disterate. Sections 1μ to 2μ thick were cut with a glass knife, placed on a slide, spread with a drop of xylene, and dried on a warming tray. They were mounted in light paraffin oil and studied with the phase and fluorescence microscopes.

The following methods were employed for altering aqueous flow. Rabbits were given an injection intravenously of a solution of Shigella endotoxin,* 6 mg. per kilogram, which produced after some hours marked hyperemia of the anterior uvea accompanied by an aqueous flare, indicating the appearance of serum proteins in the aqueous humor. In other preparations, a small draining fistula was made at the limbus with a keratome and scissors, and was kept open by repeatedly removing the fibrin which formed in it.

In order to trace the route taken by material from the blood stream to the posterior chamber or the fate of the material present in the posterior chamber, Thorotrast was injected either into the blood stream or directly into the posterior chamber. The minute electron-dense particles of thorium dioxide (Thorotrast) allowed these pathways to be visualized at high magnifications with the electron microscope. Thorotrast (0.1 ml.) (Testagar & Co., Detroit, Mich.) was injected into the posterior chamber or 15 ml. was injected intravenously.

*Prof. Seymour P. Halbert supplied this preparation.

Results

Two striking features of the adult epithelial cells facing the posterior chamber are that their free surface is lined with the internal limiting membrane which is formed by the condensation of zonula fibers and that they possess two types of surface membrane elaborations. Figure 1 shows two highly elaborated membranes forming the characteristic interdigitated margins between two epithelial cells. The intercellular space between the interdigitations varies in size and is clearly seen in this Figure. Figure 2 shows infoldings of the membrane at the free surface which faces the posterior chamber. These infoldings or clefts are always present, but their number and distribution vary. The internal limiting membrane, which lines the surface of the epithelial cells, can be seen in both Figures 1 and 2.

The ciliary epithelium of the newborn rabbit has a much simpler organization of the cell membranes than that of the adult. The cell has no elaboration of the surface membrane into the deep infoldings characteristic of the adult, nor are the complicated interdigitations between cells present (Fig. 3). The interdigitations were first found in animals seven days after birth, at which stage they were extremely simple, as seen in Figure 4. Well-defined infoldings of the surface of the cells did not appear until after the 7th day; therefore, it is apparent that the membrane elaborations may be distinguished with reference to the time at which they develop and, with the physiologic activity of the cell and of the ciliary process, at different stages of development.

After intravenous injection of Thorotrast, the lumen of many capillaries in the connective tissue of the ciliary processes were found to contain the thorium particles, and particles were also found in vesicles in the endothelial cells. In material fixed at longer intervals after the injection, thorium particles were found in the connective tissue stroma and in membrane-bound vesicles of the cytoplasm of connective tissue cells (fibroblasts and macrophages); but even for extended periods (one hour) after the injection, no particles were found in or between the ciliary epithelial cells. The capillaries of the ciliary process were found to be unusually large in diameter and to possess very thin walls. In addition to the thinness of their walls, they were characterized by the presence of pores of considerable magnitude (Figure 5). The very thin endothelial walls and pores strongly suggest that these capillaries are permeable and regularly engaged in rapid transfer of material. Similar thin-walled capillaries are found in other parts of the body where fluid transfer is rapid (Bennett et al.[4]).

When thorium dioxide was injected directly into the posterior chamber, the thorium particles were found adhering to the internal limiting membrane and, in addition, deep in the clefts and infoldings. Figure 6 is of a preparation fixed 20 minutes after the injection of thorium dioxide into the posterior chamber. The particles can be seen in the infoldings and in vesicles in the cytoplasm of these cells. Thorium particles were not found in the intercellular spaces of the interdigitated cell margins.

The fact that the thorium particles were found in closed membrane-bound structures, separate and different in diameter from the infoldings, shows that posterior chamber material with included particles was taken into the cell substance. The formation of these vesicles resembles the process of pinocytosis. In the process of pinocytosis, the substances which become internalized are at first concentrated on the cell membrane (Brandt[5]).

When Shigella endotoxin was injected intravenously, and the in situ eye was examined, the blood vessels of the iris, ciliary processes, and other eye tissues were dilated. In three to four hours after endotoxin injection the aqueous humor became turbid, presumably due to the presence of serum proteins. The fine structure of the ciliary epithelial tissue, removed from eyes in which the aqueous was turbid, was altered.[1] The infoldings on the free surface as well as the interdigitations between these cells lining the posterior chamber disappeared, and the cell margins appeared smooth. However, ciliary epithelium fixed before the aqueous humor became cloudy did not show such alterations in fine structure.

Thorium dioxide was injected directly into the posterior chamber of the eyes of rabbits which had earlier received a systemic injection of endotoxin. Eyes with turbid aqueous humor, as well as eyes in which this had not yet occurred, were fixed 20 minutes after the injection of thorium. The thorium dioxide particles were not distributed as in normal eyes. In these rabbits, the altered distribution of thorium dioxide was similar in both eyes with turbid and with clear aqueous humor. Examination of the ciliary epithelium of these eyes showed few thorium particles on the internal limiting membrane (Figs. 7 and 8). No particles were found in the free surface infoldings and none in the cytoplasm.

Sodium fluoresceinate injected intravenously into a normal rabbit was clearly visible 40 minutes later in the aqueous humor, and the eye was excised as described above. Figure 9*A* is a photomicrograph showing distribution of fluorescein in such a normal eye as seen with a fluorescence microscope. Only the apical ends of the surface epithelial cells and, to less degree, the area between the two cell layers appear to contain the fluorescein. The basal part of the cell appears blue (autofluorescence) and free of the dye.

The sections from the eye with a fistulous opening show a different pattern of fluorescence. After the fistula was made, the rabbit was given an injection of sodium fluoresceinate. The dye appeared almost immediately in the aqueous humor, indicating a disruption of the blood aqueous barrier. The eye was excised five minutes after the injection of the fluorescein. Figure 9*B* shows the typical pattern in the distribution of fluorescein in such an eye. There was no apical fluorescence as seen in the normal, but fluorescein appears to outline the cell borders.

Comment

Morphological investigations may reveal the structures by which the functions of the cells are carried out. Studies with the electron microscope permit morphological investigations of extremely fine elements of cellular structure, with which the data of the physiologists may be correlated to reach a more complete understanding of the behavior of cells. Much information exists on the selective transport of materials from the blood into the posterior chamber to form the aqueous humor (Langham,[6] Kinsey,[7] Davson[13,14]). However, it has been necessary to speculate about the route by which these materials are moved, as well as how considerable quantities of fluid are transported. Such data reveal the net result of activity taking place between the lumen of large blood vessels and the posterior chamber. Palm[8] reported that the capillary wall was the greatest barrier to sodium in the blood-ocular barrier complex. The capillary in the ciliary process is shown in the electron micrograph (Fig. 5). Wislocki and Ladman[9] described similar thin-walled capillaries in the choroid plexus, which have a comparable function, to be unusually thin-walled and to possess pores. The mode of transport of materials from the capillary lumen to the connective tissue space, as indicated by particulate material, is shown to be, at least in part, transendothelial by way of vacuoles. The presence of capillary pores has been demonstrated, but their role in fluid transport has not been shown.

The amount of thorium particles which penetrated the endothelium is extremely variable. Wissig[10] failed to find thorium dioxide particles in heart endothelium. According to Hampton,[11] however, a large amount of thorium dioxide is removed from the blood plasma by the hepatic cells. It thus would appear that thorium dioxide can be used in certain systems to limited advantage in order to visualize physiological events in the electron microscope.

Thorium particles were found in the connective tissue spaces of the ciliary process, but in no case were they found to penetrate into the epithelium of the ciliary body. It may be that they might be found to do so in more prolonged experiments. There are, however, limitations to the use of systemically injected thorium—it is not in true solution in the blood, and it does have toxic side-effects. One of these which can be seen in the electron micrographs is occlusion of some capillaries by masses of injected material, presumably having been clumped by serum proteins.

Sodium fluoresceinate, in contrast to thorium dioxide, is a small molecule, water-soluble, and of very low toxicity. It readily penetrates into the posterior chamber after its intravenous injection, and its course through the ciliary epithelium can be studied with the fluorescence microscope.

In normal ciliary epithelium, fluorescein appears as caps on the surface of the cells facing the posterior chamber. It is also found to less degree in the area between the two cell layers. Just how the fluorescein becomes localized at these two sites is not readily apparent, since there is no fluorescence elsewhere in these two cell layers. However, the apical distribution of fluorescein may be due to reabsorption from the aqueous, since this pattern is similar to that seen in the electron microscope after injection of thorium dioxide into the posterior chamber.

In the eye with the open fistula, large amounts of fluorescein rapidly appeared in the aqueous. Examination of the ciliary epithelium in the fluorescence microscope shows heavy deposits only in the areas between the cells. It seems likely that the lack of fluorescent caps on the cells facing the posterior chamber results from a disappearance of the surface infoldings in the fistulous eyes.[1]

The presence of fluorescein in the area between the cells may represent the pathway by which a filtrate of blood can reach the aqueous. This may be exaggerated and, therefore, visualized more readily when the blood-aqueous barrier is nonfunctional. Earlier investigations utilizing frozen preparations revealed that fluorescein entered the aqueous by way of the posterior chamber (Seidel,[12] Davson [13]), but more precise localization of the site of entry was not possible. Von Sallmann and Dillon [15] found that T1824, undoubtedly bound to serum protein, passed between the cells. In the present investigation, the path taken by water-soluble fluorescein shows that the transport is across the epithelium of the ciliary processes, and furthermore it would appear that the pathway is essentially between the cells rather than through the mass of cytoplasm. This observation also fits well with the data suggesting an intercellular pathway for this substance.[13]

It has generally been assumed that aqueous humor is in part elaborated by the ciliary epithelial cells and secreted into the posterior chamber where it is later modified by metabolic interchange with other gross structures, such as lens, iris, or cornea.[7,14] Concepts of secretion of aqueous humor have suggested a unidirectional passage of materials only from the epithelium into the posterior chamber.[6,13] Injection of Thorotrast particles into the posterior chamber demonstrates that the ciliary epithelium has the capacity to ingest material from the posterior chamber, showing that the posterior chamber fluid, once secreted, may be subsequently modified by reabsorption of material from it.

When thorium dioxide is injected into the posterior chamber, the internal limiting membrane lining the surface epithelial cells becomes heavily impregnated with thorium particles. Two reasons suggest themselves which may explain this accumulation of thorium. Either the internal limiting membrane is an adsorption site for thorium or it may be that this fibrous membrane[1] is acting as a sieve so that thorium becomes trapped in it. If the latter instance is correct, it would suggest that there is a flow of fluid toward the cells. One factor in the penetration of the thorium dioxide particles into the infoldings could be diffusion. However, physical diffusion could also be expected to carry these particles deep into the interdigitations between the cells where, in fact, they are not found. Furthermore, when the function of these cells is modified by the injection of endotoxins or the creation of an anterior chamber fistula, such infoldings are not found to contain Thorotrast particles. These observations strongly imply a functional significance to the appearance of the thorium particles in the infoldings rather than their distribution being merely the result of simple diffusion. These data are taken to indicate that some reabsorption occurs in the area of the surface infoldings but not in the interdigitations between the cells. Whether the reabsorption is sufficient to cause a fluid flow through the internal limiting membrane and into the surface infoldings cannot be ascertained at this time.

When normal aqueous flow is altered by the systemic injection of endotoxin, the cytoplasm of the surface cells becomes filled with small vesicles which presumably arise from the breakdown of the membrane elaborations.[1] When thorium is injected into the posterior chamber of such an eye, there is less thorium found adhering to the internal limiting membrane than is found in a normal eye (compare Figs. 6 and 7 with Fig. 5). No vesicles containing thorium are found in the cytoplasm, nor are any thorium particles seen in the remaining infoldings. This change in the distribution

of the thorium may be a reflection of a hindrance of reabsorption.

Since no thorium is found in the remaining infoldings (Fig. 7), it appears likely that these infoldings have lost their ability to reabsorb fluids from the aqueous, whereas if they were filled by diffusion one would expect to find granules in them. The subsequent breakdown of the infoldings, as shown in Figure 8, may, therefore, indicate a further diminished reabsorptive capacity of the cells.

The structural changes in the developing ciliary epithelium are strong evidence linking the membrane elaborations with the production of aqueous humor. It is significant that the interdigitated margins characteristic of the adult appear first in 7-day-old animals. Their appearance coincides with the time of initiation of aqueous humor secretion in this species, as described by Kinsey.[16] He found that ascorbic acid first appeared in amounts greater than that found in the blood plasma at about this age. This aqueous/plasma ratio gradually approached that of the adult, just as the first relatively simple interdigitations appeared at this time and gradually reached their extremely complex adult pattern. It is also of significance that the interdigitations appear prior to the infoldings of the cell membrane on the free surface. If, as has been suggested, the interdigitations are primarily concerned with secretion into the posterior chamber and the apical infoldings with reabsorption for modification of the provisional aqueous, the appearance of the interdigitations first and the surface infoldings later would seem consistent with this view.

Summary

The elaboration of the cell membranes of the ciliary epithelium is clearly associated with the production of aqueous humor.

The structure of the capillaries in the ciliary process is adapted to the transfer of fluid by the thinness of their walls and occasional pores. Evidence was obtained to indicate that fluid transport takes place, at least in part, through the endothelial cell.

Intravenously injected sodium fluoresceinate apparently enters the aqueous humor by passing between the epithelial cells of the ciliary processes or in an area closely associated with the cell interfaces and not through the central portion of their cytoplasm.

The apical infoldings of the surface cells of the ciliary process are not necessary for the penetration of fluorescein into the posterior chamber. They may be involved in the process of reabsorption of posterior chamber contents.

When thorium dioxide is injected into the posterior chamber, large numbers of thorium particles are found adhering to the internal limiting membrane as well as in the infoldings. Many vesicles containing thorium are found in the cytoplasm. When the blood-aqueous barrier is nonfunctional, as after endotoxin treatment, no thorium is reabsorbed by the epithelial cells of the ciliary processes.

The characteristic interdigitated boundaries between the surface epithelial cells were not found to be involved in reabsorption of the thorium particles.

630 W. 168th St. (32).

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Electron Microscopic Studies of the Human Eye

II. Study of the Trabeculae by Light and Electron Microscopy

L. K. GARRON, M.D., and M. L. FEENEY, A.B., San Francisco

Salzmann,[1] in 1912, published his classic text on the anatomy and histology of the eyeball, summarizing existing knowledge and describing the results of his own investigations. Little has been added to his description of the trabecular meshwork until recent years.

The trabecular meshwork has been considered to be composed of a uveal and a corneoscleral portion. The uveal meshwork borders the anterior chamber and its netlike fibers extend in an arc from the surface of the corneoscleral meshwork to the root of the iris. The meshwork is loose with large and irregular openings; the fibers appear rounded in cross section. The corneoscleral meshwork comprises the major portion of the trabeculae. The trabecular meshwork roughly is ring-shaped and in cross section is somewhat triangular, the apex being at the end of Descemet's membrane and the base of the triangle extending along a line between the scleral spur and the sclera just posterior to Schlemm's canal. It extends circumferentially around the limbus and its inner aspect borders the angle of the anterior chamber. The total volume of the trabecular meshwork has been estimated to be in the neighborhood of 4 cu. mm.

The illustrations for this article will be found in the special section of illustrations, pp. 1067 to 1076.

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Most authorities[1,5,6,7] agree that the trabecular meshwork is made up of a series of superimposed, anastomosing, flat lamellae running circumferentially around the eye at the inner limbus. Thus, the trabecular meshwork of lamellae, by their interconnections, form a triangular structure in cross section with only 2 or 3 trabecular layers being present near Schwalbe's line; whereas, at the level of the scleral spur and Schlemm's canal, there are 12 or more layers. The trabecular sheets have many perforations, the openings being mostly oval in shape with the long axis of each passageway parallel to Schlemm's canal (Fig. 1). The trabecular openings are so disposed in each succeeding layer that the stomata connect by tortuous pathways via the intertrabecular spaces. It is generally agreed that the sizes of the passageways through the trabecular sheets decrease as Schlemm's canal is approached. Theobald,[2,3] using serially sectioned eyes and reconstructions, showed that there were open pathways through the meshwork from the anterior chamber to the canal of Schlemm. Perfusion studies[4] under controlled pressures have further strengthened the thesis that open communications exist between the anterior chamber and Schlemm's canal.

The studies of Rochon-Duvigneaud,[5] Asayama,[6] Salzmann,[1] and, more recently, Ashton, Brini, and Smith,[7] have established that the central collagen core in the trabecular sheets is oriented circularly about the limbus. Since the collagen fibers form the basic "skeletal system" for the trabecular sheets, the question as to their direction and orientation is important when one considers the trabecular anatomy. These authors have found that anterioposterior sections of the

meshwork show the collagen fibers to be cut in cross section, whereas tilted frontal sections of this structure show the collagen fibers to be cut along their long axes. In spite of this clear-cut evidence in support of their observations, it is interesting that an alternate view is held by other workers. Redslob,[8] François,[9] and Wolff,[10] apparently believe that the meshwork is made up of a series of anastomosing rod-shaped fibers, radially disposed, extending from the region of the scleral furrow anteriorly to Descemet's layer of the cornea.

The histologic structure of the corneoscleral trabecular fibers was described in detail by Salzmann, based principally on observations from sections subjected to orcein and Van Gieson staining. Ashton and his associates[7] have recently repeated these studies and their findings are in agreement with those of Salzmann. By light microscopic techniques the corneoscleral fiber is described as a four-layered structure. Innermost is the central collagen core which is covered by an elastic layer. The next outermost layer is a Descemet-like glass membrane considered by many authorities to be a continuation of Descemet's membrane of the cornea because of its morphological and staining properties. The fourth and outermost layer of the trabecular tissue is the endothelium which is thought to be a direct continuation of the corneal endothelium. It covers the trabecular surfaces completely.

We have reported[11] observations on the anatomy of the trabecular meshwork studied with the electron microscope, and have confirmed the existence of an endothelial covering on the trabecular surfaces and the presence of a central collagen core. The space between the central collagen core and the endothelium was found to be composed of a "homogeneous matrix" (probably ground substance) containing irregularly shaped clumps of material having a 1,000 A. periodicity. Fine fibrils without periodicity also were found to be dispersed in this matrix. We were unable to identify elastic tissue except in scattered areas near the scleral spur. A typical Descemet-like membrane could not be found to exist beneath the endothelial layer. Other histologic and anatomic features were observed in the trabecular meshwork which seem worthy of discussion. These initial studies suggest that the conventional concept of a four-layered trabecular structure is not entirely accurate. This study is a further attempt to clarify some of the discrepancies.

Methods and Materials

The tissues used in these studies were derived from human eyes enucleated either post mortem or at surgery for melanoma of the choroid. The anterior portions of the eyes used were essentially normal, and in none was there clinical evidence of inflammation or glaucoma. From a total of 16 eyes processed and studied, 12 were surgical. The Table shows the time interval between death or enucleation and fixation of each specimen. None of the eyes was obtained from a patient less than 34 years of age; therefore, our findings may not be those which would occur in younger age groups.

To accomplish rapid fixation, the aqueous humor was aspirated and the anterior chamber gently perfused with cold osmic

Human Eyes Used in This Study

Patient No.	Age	Surgery or Post Mortem	Time Interval Between Death or Enucleation and Fixation in Minutes
490	44	P.M.	45
523	48	Melanoma of eyelid	1
564	40	P.M.	24
565	66	Melanoma of choroid	5
609	67	P.M.	18
610	67	P.M.	21
648	51	Metastatic carcinoma of the macula	5
706	51	Melanoma of choroid	11
707	59	Melanoma of choroid	2
754	67	Orbital tumor	2
805	44	Melanoma of macula	2
811	65	Melanoma of choroid	2
812	62	Melanoma of choroid	10
814	72	Melanoma of choroid	1
838	76	Melanoma of choroid	1
857	34	Hemangioma of choroid	2

acid. Radial strips of limbal tissue, including the trabecular meshwork, were excised and further fixed in osmic acid, embedded in methacrylate, and thin sectioned for electron microscopy.[11] In most of the eyes, the trabecular meshwork was studied by light and phase microscopic techniques using 1μ thick sections cut in the three principal planes as shown in Figure 2.

Formalin fixed, celloidin embedded tissue was also studied, being obtained from routine laboratory specimens in which the anterior segment was normal. To accomplish this, a small portion of limbal tissue was excised from the original celloidin block, remounted, and cut in 5μ or 6μ sections. These were subjected to a variety of stains and studied with a light microscope. In this way, it was possible to repeat some of the investigations of those who had used conventional histologic methods and to compare these findings with those of electron microscopic techniques.

Results

1. *Histologic Studies.*—Methacrylate embedded tissues were cut in the meridional and tilted frontal planes in 1μ thick sections and subjected to a variety of staining techniques. Conventional staining techniques were not found to be adequate in osmic acid fixed, methacrylate embedded material. Prolonged staining in concentrated stains was used, but there was considerable variation in the staining of consecutive sections even after the methacrylate was removed. Elastic tissue stains were not specific, and all conventional differential staining methods were found to be unsatisfactory.

A new staining technique[12] using periodic acid-Schiff, hematoxylin and fast green proved useful on these thin sections with a differential staining of constituent portions of the trabecular tissue. When examined with a light microscope, the central collagen core stained a bright green, the endothelium pink, and the nuclei bluish-green. In the space between the core and the endothelium, the background color was light green in the inner and central trabecular zones and was characterized by the presence of numerous dot-like dark green granules (Fig. 3, *CC*), of the same intensity as the collagen core. In the tissue near Schlemm's canal, the material beneath the endothelium varied in staining from pale green to pale pink and the dot-like granules were rarely seen.

After examining a number of sections treated with this stain, it was possible to note that the collagen core was thickest and most prominent in the central and inner trabecular sheets (Fig. 4). As one approached Schlemm's canal, the collagen fibers in the trabeculae were found to decrease in numbers and frequency. Immediately adjacent to Schlemm's canal the tissues took a pale pink color suggesting that collagen was absent. This tissue appeared much more compact and less sheet-like than the inner trabeculae. At high magnification this tissue showed numerous endothelial cells. Thickness of this pale staining cellular zone varied considerably in different areas of the same section (Fig. 5).

Some of the endothelial cells lining the inner wall of Schlemm's canal showed vacuoles in their cytoplasm similar to those seen previously by electron microscopy (Fig. 6). A number of red blood cells were found in Schlemm's canal and also in the intertrabecular spaces throughout the network (Fig. 3). In one fortuitous case, a red blood cell was found incarcerated in a passageway between Schlemm's canal and the trabecular tissue (Fig. 7), suggesting that the other red blood cells in the intertrabecular spaces had been drawn out of Schlemm's canal at the time of aqueous aspiration.

Formalin fixed, celloidin embedded tissues were cut 5μ to 6μ thick in the three principal planes. These sections were processed with a variety of stains. For our purposes we found tissues stained with Weigert's elastic tissue stain and counterstained with the Shorr stain to be the most

satisfactory for the demonstration of the trabecular components. In the central trabecular area the collagen core stained blue-green and the endothelial zone reddish-brown. Numerous dark brown granules in a lighter background were dispersed between these two. The collagen core thinned gradually toward Schlemm's canal and in the tissue immediately adjacent to the canal there was a lack of the blue-green staining collagen. As noted in the methacrylate embedded sections, there was considerable variation in the width of this cellular zone in different sections. The endothelial cells on the inner wall of Schlemm's canal showed numerous cytoplasmic vacuoles (Fig. 8).

2. *Electron Microscopic Studies.*—Uveal Meshwork: The open net-like structure of the uveal meshwork causes it to present a round profile regardless of whether the meshwork is cut in the tilted frontal or meridional plane (Fig. 9). The uveal fibers are 5μ to 12μ in diameter. They are composed of collagen fibers which pass through the center parallel to the long axis of the uveal fiber regardless of its direction in the eye, i.e., the collagen core does not maintain the circumferential orientation which is exhibited in the corneoscleral portion of the meshwork. The collagen is compact in the core and there are irregular deposits of dense amorphous "cement substance" around groups of collagen fibers. The core appears to be circumscribed by some circularly disposed collagen fibers which are likewise embedded in a cement-like material. More peripherally is a wider zone of light density which contains loosely dispersed collagen fibers which run circularly, obliquely, and longitudinally. The periodicity of these fibers varies between 400 A. and 600 A. There are irregular deposits of dense amorphous material in this area also which, when treated with uranium acetate, occasionally reveal a periodicity of dark bands spaced 1,000 A. apart. This material is not a prominent feature of the uveal fibers, however. There is a medium dense, finely granular basement membrane beneath the endothelium. The endothelial cytoplasm varies in thickness from 0.3μ to 2.0μ and may contain pigment granules. The endothelium of uveal and corneoscleral fibers frequently shows cytoplasmic junctures. The cytoplasmic components appear to be similar to those seen elsewhere in the trabeculae.

Corneoscleral Meshwork: The component parts of a typical corneoscleral trabecular sheet have been partially reported.[11] Figure 10 and Figure 11 show the sheets cut in two planes; the meridional, with the collagen core cut in cross section, and the tilted frontal, with the collagen core cut longitudinally.

Figure 12 shows a tilted frontal section through a portion of a trabecula. The collagen core is sectioned longitudinally and the fibers show the usual banding of approximately 640 A. In Figure 13, the plane of sectioning is meridional and the core is cut in cross section. The individual fibers measure about 400 A. in diameter and are embedded in a rather dense, finely granular substance. The core varies in width from 1μ to 4μ. Tilted frontal sections show the core to taper to a point at the site of a trabecular opening (Fig. 14). Actually the collagen fibers are cut diagonally as they digress from an essentially circumferential direction around the eye to pass above or below the elliptical holes of the sheet.

The core is ensheathed by a layer of thicker fibers measuring 0.14μ to 0.2μ in diameter (Fig. 15). These fibers have a periodicity of 1,000 A. There is an amorphous light density matrix between the core and the basement membrane of the endothelium. In this matrix, in addition to ground substance, are two components: very fine fibrils approximately 100 A. in diameter which have no demonstrable periodicity, and irregular clumps of a material with 1,000 A. banding.

As the sections approach Schlemm's canal, there is a change in the appearance of the sheets and the component parts. The

collagen core has disappeared as a true core, becoming arranged in thin bundles or unoriented fibers. The clumps of 1,000 A. banded material are scarce.

Inner Wall of Schlemm's Canal (Pore Tissue [13]): In a zone varying from 2μ to 15μ in thickness adjacent to Schlemm's canal, the tissue appears less sheet-like than the corneoscleral lamellae. This pore tissue is made up essentially of endothelial cells and ground substance which follow the irregular contours of the canal and therefore is not parallel to the sheets. It is more compact in the sense that intertrabecular spaces are lacking. Asayama [6] was the first to note and describe in detail the unique characteristics of this tissue.

The endothelial cells lining the inner wall of the canal and elsewhere in this zone lack a basement membrane in the usual sense, but rather lie on a loosely amorphous ground substance of variable thickness (Figs. 16 and 17). In some areas the endothelial cytoplasm is unsupported by ground substance, there being an empty space directly beneath the cell membrane. Elsewhere, the underlying ground substance appears moderately electron dense. Numerous cytoplasmic "vacuoles" are seen in the endothelial cells lining the inner wall of the canal. Most of these vacuoles measure 2μ to 5μ in diameter, although occasional ones are as much as 8μ wide (Fig. 18). The cytoplasm around these spaces is frequently so finely attenuated that only the double cell membrane is apparent. With our techniques these vacuoles seem to be empty.

The ground substance beneath the endothelium varies considerably in density in different areas. Often it thins out to an empty space between cytoplasmic boundaries. The ground substance appears fibrillar when treated with phosphotungstic acid (Fig. 19).

In our sections, we have been unable to show an open pathway across the pore tissue; however, in Figure 20, a red blood cell may be seen incarcerated in an opening from Schlemm's canal. It appears from this section that the red blood cell was trapped in this position during aspiration of aqueous from the anterior chamber.

Scleral Side of the Canal: The endothelial cells lining the scleral or external side of Schlemm's canal do not have the large vacuoles seen on the inner wall, although smaller ones are occasionally seen. The basement membrane, unlike that of the inner wall, is prominent, medium dense, and measures 0.15μ to 0.3μ in width (Fig. 16).

Comment

In the study of the structural anatomy of the trabecular meshwork by electron microscopic techniques, it is difficult to correlate some of the findings obtained with those generally obtained by conventional histologic methods. "Elastic fibers" and the "Descemet-like membrane" represent two of the trabecular components which are observed by light microscopic methods, yet are not identifiable as such with the electron microscope.

Repetition by us of the work done by Salzmann, and by Ashton and his associates, confirms their observation that there is a component in the trabecular meshwork just external to the collagen core which stains with the usual elastic tissue stains, and that there is a component beneath the endothelium which stains more or less as Descemet's membrane with a variety of stains including periodic acid-Schiff and Van Gieson. In addition, we found that this zone also stained with the usual elastic tissue stains such as orcein, resorcin-fuchsin and aldehyde-fuchsin.

However, with tissues fixed in osmic acid, embedded in methacrylate and cut 0.5μ to 1μ thick, the above findings were only partially duplicated. Tissues processed in this manner react poorly to the usual histologic stains; however, these "thin" sections were ideal for study by phase microscopy where intense staining is not mandatory.

One micron thick sections which are viewed by phase microscopy show granules or dots in the area between the collagen core and the endothelium in the position

ordinarily assigned to the "elastic fibers". These granules are identifiable with the clumps of 1,000 A.-banded material seen with the electron microscope. It is our impression that the "elastic fibers" seen by conventional histologic methods, the granules seen by phase microscopy, and the 1,000 A.-banded material seen by electron microscopy are one and the same.

Our present knowledge of the structure and appearance of elastic fibers by electron microscopy indicates that these 1,000 A.-banded figures cannot be considered elastic fibers despite their affinity for elastic tissue stains. Recent studies indicate that elastic stains are not specific for elastic tissue; in fact, certain mucopolysaccharides and even collagen [14] are frequently stained. Since both elastic tissue and collagen have mucopolysaccharide components, this is not surprising.

There is additional evidence that this banded material is not elastic tissue but actually belongs to the collagen class of connective tissue. Dr. Marie Jakus [15] has shown by x-ray diffraction studies that Descemet's membrane of the cornea is collagenous tissue. The banding observed in Descemet's membrane when the sections are cut perpendicular to the plane of the membrane coincides with the banding of clumps of material in the trabecular sheets. Descemet's membrane in the peripheral cornea near the trabeculae not only shows the structure reported by Jakus in the outer one-third of the membrane, but also shows the irregularly shaped 1,000 A.-banded figures throughout the inner portion as well.[16] Their appearance is identical with those in the trabecular sheets.

The foregoing discussion would seem to be an argument for identifying the histologist's "Descemet-like membrane" of the trabeculae with the 1,000 A.-banded figures seen by electron microscopy. It is our opinion that the zone between the collagen core and the endothelium (the homogeneous matrix) represents the "Descemet-like membrane" seen by light microscopy. It should be remembered that Descemet's membrane of the cornea may stain positively with either the elastic tissue or the mucopolysaccharide stains, but always more intensely than the "Descemet-like membrane" of the trabeculae. The zone between the collagen core and the endothelium of the trabecular sheet, which stains similarly to Descemet's membrane, consists of a mat of ground substance which is probably a mucopolysaccharide or mucoprotein. It is also likely that the basement membrane beneath the endothelium is a condensation of this same type of material. Therefore, because of their basic similarity in chemical composition the staining reactions of the materials in these two locations would be expected to be similar.

Since reporting the presence of vacuolated endothelial cells on the inner wall of Schlemm's canal (1958), this observation has been verified in histologic specimens, as well as in additional specimens viewed with the electron microscope. Speakman [17] has suggested that these findings represent post mortem changes. He was able to demonstrate the presence of cell vacuolization after rapid fixation of eyes in the endothelium of the cornea and in the inner wall of the canal. He was able to show that this change also took place very rapidly in unfixed specimens. We agree that vacuolization of the corneal endothelium does occur as a result of delayed fixation. Under the electron microscope we have observed marked microvacuolization of the endothelium in postmortem eyes which were not fixed for four or more hours after death. However, in all of the eyes discussed in the present report, the cytoplasm of the corneal endothelium showed no vacuolization or other apparent postmortem change and the mitochondria and endoplasmic reticulum were well preserved. Furthermore, the cells lining the canal in these same eyes, while also showing excellent preservation of cytoplasmic elements, exhibited these "vacuoles." We are, therefore, convinced that the "vacuoles" on the inner

wall of the canal are not artifactual or the result of postmortem change.

Some doubt has been expressed as to whether these "vacuoles" are vacuoles at all. Holmberg [15] made serial sections through these cells and reported that these spaces were formed by cytoplasmic projections of adjacent endothelial cells. He was able to trace the corridors formed by these cells into the ground substance of the pore tissue and also internally to the intertrabecular spaces.

Summary

Normal human trabecular meshwork has been studied by means of the following techniques:

1. Formalin fixation, paraffin and celloidin embedding, 5μ to 6μ sections, several special stains, and light microscopy.

2. Osmic acid fixation, methylacrylate embedding, 1μ thick sections, several special stains, and light and phase microscopy.

3. Osmic acid fixation, methylacrylate embedding, 0.05μ to 0.025μ sections, and electron microscopy.

Using the techniques listed above, we have attempted to correlate the findings obtained by light microscopy with those obtained by electron microscopy.

The typical electron microscopic appearance of the uveal meshwork, the corneoscleral meshwork, and the tissue bordering Schlemm's canal is presented.

In the corneoscleral trabecular sheet, the "elastic fibers" seen by conventional histologic methods were identified as the clumps of 1,000 A.-banded material seen in the homogeneous matrix by electron microscopy. Evidence is presented which indicates that this material is not elastic tissue.

That portion of the corneoscleral trabecular sheet which stains similarly to Descemet's membrane appeared to be the homogeneous matrix seen by electron microscopy. Structurally this matrix differs from Descemet's membrane, but both are thought to contain mucopolysaccharides.

As first reported by Asayama, the tissue immediately adjacent to the inner wall of Schlemm's canal differs from the corneoscleral meshwork. Electron microscopically it was composed of endothelial cells and ground substance.

Evidence is presented indicating that the "vacuolizations" of the cells along the inner wall of the canal are not due to artifact or postmortem change but are normal characteristics of this endothelium.

Francis I. Proctor Foundation, University of California (22).

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Ultrastructure of the Iris: The Intercellular Stromal Components

A. J. TOUSIMIS and BEN S. FINE, M.D., Washington, D.C.

The fine structure of the iris (rhesus monkey and man) has been studied by electron microscopic examination of ultrathin sections.[1] The results reported were in general agreement with those based on classical histologic methods [2,3] and revealed in greater detail the structure of the cells, blood vessels and nerves of the stroma, the muscles, and the cells of the pigment epithelium. Such sections (about 0.025μ in thickness) are most suitable for the study of cellular structures, i.e., the structures composed of an abundance of macromolecular aggregates (microparticles, membrane-structures, and filaments), but they provide very little information about components in low concentration. A good example of such components is found in the intercellular substances of the loose iris stroma. The technique of ultrathin sectioning has revealed the presence of collagen fibrils between the stroma cells, but their distribution and orientation with regard to other tissue components was not clear.

To obtain a better appreciation of the relationship of the intercellular substances to each other and to the larger structures around them, we have used somewhat thicker sections, prepared as outlined in Materials and Methods.

The illustrations for this article will be found in the special section of illustrations, pp. 1077 to 1087.

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Materials and Methods

Irides from 18-month-old rhesus monkeys, a 44-year-old Negro man and a 2-year-old white girl were placed in cold (0 C-4 C) 1% osmium tetroxide suspended in isotonic veronal buffer (pH 7.35), dehydrated in graded ethanol, and embedded in methacrylate according to the procedure described elsewhere.[1] Sections approximately 0.1μ in thickness were made with a Porter-Blum [4] ultramicrotome, using glass knives. Sections floated on a 10% acetone-water trough, and then were placed on carbon-film on formvar-covered grids.[5] The methacrylate in these sections was dissolved with xylene, leaving the tissue on the carbon film. The sections were then shadow-cast with uranium at arctan 1/3 followed by carbon lightly at arctan 1/1.5. Electron microscopy was carried out with an RCA (Model-EMU-3B) instrument equipped with a Canalco condenser aperture and a 40μ diameter objective aperture. Electron micrographs were made at magnifications of 1,700 to 14,000 diameters, and further enlarged photographically for their study or for the illustrations in this report.

Results and Comment

Collagen fibrils of 600 A. average width and 550-600 A. axial periodicities were found between the cellular components of the iris stroma, as reported elsewhere in our study of ultrathin sections.[1] Similar fibrils were observed in both monkey and human irides (Figs. 2-4). Their axial periodicities are the same, and these fibrils show typical collagen interbanding. Fibrils shown in these electron micrographs do not appear uniform in width or banding. This has

been interpreted as an artifact due to incomplete removal of methacrylate and relative orientation with respect to the source of the shadowing material. The variation in length of the collagen fibrils shown in these sections suggests their random orientation. The fibrils vary from almost perfect cross-sections, which appear as small particles of the same width as those sectioned longitudinally, to those oriented longitudinally in the plane of the section.

In thicker sections (Figs. 5-8), the fibrils appear to be aggregated (with random orientation) when near stromal cells, nerves, or capillaries. This leads to a grouping of cells, fibrils, and capillaries, or cells, fibrils, and nerves. The presence of collagen fibrils in the stroma could have functional significance in giving support to stromal cells, capillaries, and nerve fibers. During dilatation or constriction of the pupil, the structural organization of the iris is changed; thus the bundles of stromal components can not have a rigid configuration. It is possible that the random orientation of collagen fibrils about stromal cells, vessels, and nerves is essential for maintaining support of these structures while permitting wide latitude in change of position during rapid iris movements.

Because of the groupings of stromal cells, fibrils, vessels, and nerves, bundle formations are seen. Between these bundle formations are numerous channel-like "spaces" containing virtually no collagen fibrils (Figs. 6-10). Such channels have been observed to be continuous with numerous apertures present on the iris anterior surface border (Figs. 11-14). The anterior surface of the iris in monkey and man consists of loosely aggregated stromal components (pigmented and nonpigmented cells, and occasionally, capillaries and collagen fibrils). Anterior surface apertures have been defined as portions of that surface which do not contain cellular components; however, collagen fibrils are present in low concentration. The collagen fibrils in the anterior iris surface could have the following two functions: (a) provision of additional support to the cells in this layer, and (b) formation of a sieve-like structure within the apertures.

Both light microscopy and low magnification electron microscopy demonstrate aggregates of stromal cells oriented in planes passing from the periphery towards the pupil, and from the posterior layers towards the anterior surface. Cells forming these aggregates do not interdigitate, but are loosely held by their long cytoplasmic processes, collagen fibrils, and possibly by other intercellular substances.

The statement is frequently made that the iris vessels are characterized by a thick adventitia and that the arteries possess a thin muscularis.[2] Electron microscopy of ultrathin sections[1] has revealed only capillary type iris vessels, the walls of which consist of a single layer of endothelium. Surrounding these capillaries are collagen fibrils and/or cells which appear identical with those characteristic of the iris stroma (pigmented and nonpigmented) (Figs. 5,6, 8,9,10,15,16). Vessels were similar in both the human and the monkey. Blood cells were found "suspended" in the center of the lumen, which suggests the effectiveness of the fixation used in these studies. The plasma appears granular in shadowcast preparations.

In addition to the intercellular collagen fibrils, a fine granular and/or filamentous substance has been found in the iris stroma (Figs. 4,6,7,8,10,15,17,19,20). The granular material is interpreted to be drying patterns of a macromolecular substance. The filaments appear to be beaded and measure up to 200 A. in width (Fig. 18). A hyaluronidase-sensitive acid mucopolysaccharide has been demonstrated in the human iris by histochemical methods and light microscopy.[6] Whether the substance observed in our electron micrograms is tropocollagen—at least the filamentous form—or a mucoprotein cannot be concluded from our present studies.

Large bundles of nerve fibers with stromal cells and collagen fibrils have been found throughout the iris stroma (Figs. 1, 17, 18). The axoplasm of these nerve fibers contains numerous filaments, about 100 A. in width, oriented in the direction of the long axis of the nerve (Figs. 21 and 22). Long mitochondria about 0.15μ in diameter, also oriented in the direction of the nerve fiber, were observed in the axoplasm.

Summary

The electron microscopic study of tissue by means of ultrathin (circa 0.025μ) sections gives detailed information of cellular and intracellular structure where the macromolecular aggregates are relatively dense. Much less information is obtained about the intercellular components, particularly where they are in low concentration, as in the loose iris stroma. Use of shadowcast "thick" sections (circa 0.1μ) in this study was rewarded by a clearer appreciation of the intercellular substances in the iris stroma. The "empty" spaces of the ultrathin sections were found to be occupied by large numbers of typical collagen fibrils. The "spaces" between these fibrils also appeared to be empty, but the shadowcast drying patterns of a macromolecular material could be easily found in these preparations in many areas. Whether these interfibrillar macromolecular substances are a mucopolysaccharide cannot be determined from the present study.

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Armed Forces Institute of Pathology, Walter Reed Army Medical Center (25).

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Early Changes in the Lens Epithelium After Roentgen Irradiation

A Correlated Light and Electron Microscopic Study

THEODOR WANKO, M.D.; LUDWIG von SALLMANN, M.D., and MARY ANN GAVIN, M.S., Bethesda, Md.

Introduction

The ultrastructure of the normal lens epithelium has only recently been described.[1] On the basis of this report it was possible to investigate the cytopathology of this cell layer in various forms of experimental cataracts with the electron microscope. It is of particular interest to follow the effects of ionizing radiations, since it has been shown that injury to the epithelium is an important pathogenetic factor in the development of radiation cataract.[2-6] Previous light microscopic studies on whole mounts of lens epithelium have provided quantitative data on incipient radiation damage to the cell nucleus and established the time sequence of such changes on a cellular level.[4-6]

In the present work, changes of the fine structures of the lens epithelial cells induced by an ionizing radiation are described at selected time intervals and correlated with results of light microscopic examinations.

Material and Methods

Both eyes of eight young albino rabbits (1.5 to 2.5 kg.) were irradiated individually with 1,500 r x-rays. The radiation factors were as follows: 220 kv., 20 ma., 119 r per minute in air, 0.25 mm. copper and 0.5 mm. aluminum filtration, 15.0 cm. target skin distance, 20 mm. field (round). The animals, under pentobarbital sodium (Nembutal) anesthesia, were positioned so that one eye could be irradiated while the other was shielded and remained outside the beam. The irradiated eyes were examined for early lesions with the biomicroscope. Two animals of the series were killed at each of the following time intervals: 24 hours, and 4, 10, and 14 days after irradiation. The left eyes were processed for the Feulgen reaction, and whole mounts of the epithelium were prepared, following the established procedures.[7] For electron microscopic study, the lenses were removed from the right eyes in situ, immediately fixed, and processed as outlined previously.[1] From each lens, 15 to 20 small tissue blocks were excised according to zones. Electron micrographs were recorded at magnifications ranging from × 3,350 to × 21,500 with an RCA EMU-3C model and photographically enlarged from three to four and one-half times.

Results

Biomicroscopically, mottling or fine stippling of the corneal epithelium, a faint flare, and a moderate number of cells in the aqueous humor are seen at the 24-hour and 4-day intervals. These changes subside 10 to 14 days after irradiation.

The results of light microscopic examination of flat mounts of the epithelium at the stated time intervals after irradiation are

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From the Ophthalmology Branch, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

TABLE 1.—*X-Ray Irradiated Albino Rabbits (1,500 r)*

Rabbit No.	1	2	3	4	5	6	7	8
Days after irradiation	1	1	4	4	10	10	14	14
Mitoses	5	2	0	10	158	56*	313	410
Degenerate cell clumps	140	42	20	171	236	11	160	181

* Possibly faulty technique.

recorded in Table 1. Since in the present series both eyes were irradiated, no controls are available. Therefore, the average of the mitotic counts in 515 normal adult rabbits of previous experiments is used as a reference value.[8] It can be seen from the Table that mitotic activity in the lens epithelium of the irradiated eyes is suppressed during the first 4 days, has recovered at the 10th day, and shows an increase of about 100% at the 14th day. As usual, the number of degenerate cells greatly varies from animal to animal at the early postirradiation intervals. The reasons for these variations have been explained in previous studies.[5] The number of mitoses and degenerate cells in the preparation of Rabbit 6 are so low, however, that most likely this eye did not receive the calculated dose, due to faulty technique. The cell pathology follows the usual pattern which has been established in many observations in early radiation experiments on the eye.[4,5] The fine granularity of the nucleus is generally preserved in early stages, but in a number of cells the small Feulgen-stained elements become coarser and form clumps, first in the nucleoplasm, and later in the cytoplasm, where they are often surrounded by an unstained halo. When such clumps further coalesce, they frequently indent the adjacent part of the cell nucleus (Fig. 7). This extruded, clumped chromatin, in some instances, may represent a small loss of nucleoplasm and not interfere with the survival of the cell. In others, this phenomenon may precede extensive karyorrhexis. These deeply purplish-stained, homogenous clumps differ from other small, Feulgen-stained bodies which show the same structure as the cell nucleus and are called accessory nuclei (Fig. 10). They appear to be sequestrated from protruding parts of the nucleus and to give rise to multinuclear cells which are likely to develop into giant cells. Attempts to stain cytoplasmic components in flat mounts were not successful.

Electron Microscopic Observations

Examination of irradiated lenses reveals many morphological details not observed in the normal lens epithelium previously investigated by the same method. These changes, caused by x-irradiation, occur in circumscribed areas that are often contiguous with apparently normal regions. Sometimes, severe damage is limited to one cell of an examined area. Between the first and last phases observed there is a progressive increase in the number of elements involved and in the extent to which the individual cellular constituents are altered. Some of the changes in cell organelles observed at one time interval are qualitatively comparable to changes through subsequent periods. These radiobiological effects will be described relative to the individual cell constituents and to the sequence in which they occur. Reactions to the x-ray beam are similar in the various zones of the epithelium, and, therefore, the description will refer to a particular region only in specific instances. In the stages studied the pattern of the cytoarchitectural organization within the lens epithelium and its relation to capsule and fibers remains unchanged. All the observations are briefly summarized in Table 2.

Nuclei.—At the 24-hour interval obvious changes in nuclear structures mainly concern the location and appearance of nucleoli. Normal nucleoli are often eccentrically located but well separated from the nuclear membrane by karyoplasm. In irradiated cells they are frequently seen at the periphery, immediately adjoining the nuclear membrane (Fig. 1). In addition, the dense granular constituents of the nucleolus, which normally are arranged in a regular pattern,

TABLE 2.—*Radiation-Induced Changes in the Fine Structure of Lens Epithelium*

	Days After Irradiation			
	1	4	10	14
Nuclei	Displaced nucleoli; paranuclear aggregate	Two nuclei; indistinct nuclear membranes, spongy nucleoplasm	Two nucleoli; displaced nucleoli; accessory nuclei; paranuclear aggregates	Accessory nuclei; clumped nucleoplasm; many large stomata in nuclear membrane; paranuclear aggregates
Mitochondria	Conically tapered ends	Long, lamellated formations (3μ)	Club-shaped, tapered, lamellated ends; cristae distended & displaced; granularity of matrix	Elongated, distended; granularity of matrix, club-shaped; cristae displaced & distended
Endoplasmic reticulum	----	----	Increase in quantity in equatorial zone	Increase of attached & isolated dense granules; dense lumen
Golgi complex	----	----	----	----
Low-density elements	----	----	Increase in circumscribed areas	Diffuse increase; arranged in rows
Ground substance & cell membranes	Loss in density; low opacity of membranes	Loss in density, subcapsularly	Intercellular space increased; dense, tortuous, small elements subcapsularly	Loss in density, subcapsularly

are somewhat dispersed through the surrounding nucleoplasm.

In one instance (Fig. 6) a large portion of a partially disintegrated nucleus is replaced by a heterogenous complex of rounded forms which individually contain fine, granular material of varying densities and irregularly shaped, opaque profiles (Fig. 5). This agglomerate is sharply outlined and clearly distinguishable from the nucleoplasm on one side and the fairly well-preserved cytoplasm on the other. A nuclear membrane cannot be seen at the junction between the nucleoplasm and the described aggregate (Fig. 6).

On the fourth day after exposure to the x-ray beam, binucleated cells are observed in the equatorial area (Fig. 2). Two such nuclei, differentiated from deeply indented nuclear profiles by serial sections, usually lie close to each other and lack regular, well-defined nuclear membranes. In addition, the nucleoplasm seems less dense than usual giving an over-all effect of low opacity.

Ten days after irradiation nucleoli are still seen near the nuclear border, as at the 24-hour interval. The dense nucleolar granules are scattered and lose their discrete, compact arrangement in that portion adjacent to the nuclear membrane, while, centrally, their organization into thick, distinct coils is largely maintained (Fig. 4). In other instances, two nucleoli are present and are located at opposite poles of the same nucleus (Fig. 11). In some cells the nuclear envelope appears thicker and more opaque than usual.

Occasionally, the nuclear profile, bounded by its membranes, shows several finger-like projections extending into the cytoplasm. The nucleolus may be located in one such projection (Fig. 3). In other cells the cytoplasm contains small deposits of nucleoplasm which lack a membranous boundary and are situated near the nucleus or directly adjoining its outer membrane. In such a case, the two profiles of the nuclear membrane are widely separated to a maximal distance of about 600 A. (Fig. 8).

Sometimes as many as four small nuclei are observed in a single cell. Paranuclear aggregates, similar to the one observed at the 24-hour interval, become more numerous. The dense profiles of these structures are more closely grouped or are centered around an opaque body of homogenous appearance. In contrast to the 24-hour observation, the nucleus adjacent to the com-

plex has a normal shape and possesses a well-defined boundary (Fig. 5).

Another form of nuclear response is seen in lenses 14 days after irradiation. The karyoplasm becomes condensed to intranuclear clumps which are detached from the nuclear boundary. No nucleoli are recognized in such instances. The nuclear membrane may be of normal character, or it may appear as a rather wide band of low opacity when it is sectioned in certain planes (Fig. 12). Many prominent stomata, about 500 A. in diameter, are disposed along the circumference of such a boundary. Also, some of the small masses of nucleoplasm outside the main nucleus may possess a similar, perforated contour. At this phase, some cells contain two or three accessory nuclei (Fig. 9). Their fine structure resembles that of normal nuclei; they contain profiles similar to nucleoli composed of dense, tightly aggregated granules but lack the usual arrangement in coils.

Mitochondria.—Mitochondria in normal lens epithelial cells are observed as round or oval profiles of a maximal diameter of 1.0μ. Occasionally, 24 hours after exposure, the ends of mitochondria appear conically tapered without definite increase in the over-all length.

After four days, a few thin, longitudinally lamellated structures are seen and reach a maximal length of 3μ. They are bounded by a membrane, and their ends can be either tapered or slightly distended (Fig. 15). Their identification as mitochondria rests mainly upon the presence of internal profiles similar to cristae. Observations in later phases confirm this interpretation.

Ten days after irradiation, a mitochondrion in one portion can be thin and lamellated resembling the structures seen after four days, while the other part can be enlarged to a club or hammer shape (Figs. 17, 18, 19, and 20). In these distended segments the spacing between the contours of one individual crista may measure as much as 500 A. (Fig. 21). In other instances, mitochondria exhibit an over-all increase in size and a decreased opacity of all contours (Figs. 5 and 16). Sometimes the larger mitochondria have fine, granular material dispersed in their matrix between the cristae (Figs. 5 and 21). A diffuse or partial distension of the cristae often occurs, together with their displacement toward the periphery of the mitochondrion (Fig. 16).

At the two week's interval, sections of lenses generally exhibit mitochondrial changes similar to those observed in the preceding phases. In some cells there is a considerable decrease in the number of mitochondria (Figs. 12, 13, and 14) coincident with marked radiation effects on the endoplasmic reticulum and increase of low-density elements.

Endoplasmic Reticulum.—Changes of the rough surfaced endoplasmic reticulum are first visible 10 days after irradiation. At this stage many small, vesicular profiles with attached dense granules are found in the equatorial zone, whereas they are rarely seen in this region in normal epithelium (Fig. 4).

The changes of this structure are more pronounced 14 days after exposure to x-rays. Its membranous profiles are irregular, and their intracysternal spaces large and opaque. The number of dense granules attached to membranes is markedly increased, as is the number of small clusters of isolated granules (Fig. 13). These forms are most prominent in cells which also show the nucleoplasm clumped and retracted from the nuclear membrane (Fig. 12). In addition, such cells contain few cytoplasmic components other than endoplasmic reticulum and elements of low density, as described in the paragraph on mitochondria.

No deviations are noticed in the appearance or amount of smooth surfaced endoplasmic reticulum.

Golgi Complex.—Throughout the series of irradiated lenses studied, the Golgi complex retains its usual configuration, location, and individual constituents.

Cytoplasmic Ground Substance, Low-Density Elements, and Intercellular Rela-

tionship.—In the examined material, the formed cytoplasmic elements are more widely spaced in a matrix that is less dense than usual. As a result, the structures of normally low opacity show increased contrast. This condition is most pronounced in the earlier phases and is more prominent in the subcapsular layers than in deeper areas. A similar phenomenon is noted in the adjacent lens capsule where fine lamellar profiles are discernible (Fig. 21).

Ten days after x-ray treatment filamentous structures of low density increase in number. At this time, circumscribed areas of cytoplasm in the vicinity of the nucleus as well as in the cell periphery seem devoid of other formed structures (Figs. 2 and 4). After 14 days the cytoplasm of a cell may be largely occupied by these elements, which occasionally are arranged in parallel rows. As already stated, they and the rough surfaced endoplasmic reticulum are the preponderant configurations in the cytoplasm of such cells (Fig. 13).

At the 10-day interval an inclusion is frequently observed which cannot be identified as one of the commonly recognized cytoplasmic structures. It consists of a loose aggregate of small, elongated profiles which are very dense and tortuous, ranging in thickness from 200 to 250 A. (Figs. 21, 22, and 23). They are either completely opaque and homogenous or have a less dense core. They are located in proximity of interdigitating cell processes near the lens capsule and are often closely associated with cell membranes.

In the specimens studied 24 hours after irradiation, cell membranes sometimes seem less opaque over a short distance, and after the 10-day interval, there is often an increase of intercellular spacing to 500 A. (Fig. 14).

Comment

A single exposure of the eye to the x-ray beam or to other types of ionizing radiations is thought to elicit initially a sequence of nuclear changes in the lens epithelium. Dose dependency and time course of this injury to the epithelial cells have been studied most thoroughly in adult rabbits by examining whole mounts of the monolayered epithelium subsequent to the Feulgen reaction. With this specific stain, cytoplasmic changes are noticed only if they consist of the deposition of DNA-containing material or of extensive vacuolic degeneration in later stages. Signs of nuclear fragmentation are clearly evident 24 hours after exposure to x-ray levels from 1,000 to 2,000 r. The counts of degenerate cells increase at the 10-day interval, when mitotic recovery is complete; they rise steeply two weeks after irradiation, at which time cell division is in an overcompensating phase.

The possibility of correlating the results obtained with the two methods employed in this study is limited. Such a correlation is restricted to those formations which, under the light microscope, are Feulgen-positive and which exhibit nucleoplasmic characteristics in the electron beam. No comparisons are feasible regarding cytoplasmic constituents resolved by the electron microscope and the quantitative evaluations of nuclear changes made possible by the flat-mount technique.

The electron microscopic studies on irradiated lens epithelium reveal structural changes ranging from slight alteration of individual cell elements to a total disarrangement of cellular organization. Some cells show partial disintegration and the emergence of fine structures which cannot positively be identified with any preexistent constituents. The observations indicate that with the exception of the Golgi complex all cellular structures gradually become involved in the cell responses to the irradiation.

The first detectable changes probably take place in the nuclei and find their morphological expression in the displacement and structural rearrangement of nucleolar elements. At the same time, some parts of the mitochondrial profiles assume a more filament-like shape. The significance of this

minor change is in itself doubtful, since, even under standard conditions, morphological characteristics of these particles vary in the different regions of the lens. Nevertheless, a conically tapered appearance of mitochondria might be interpreted as an initial step towards more conspicuous transformations seen at later stages. In addition to the slight changes of the formed elements, there is, at the earliest interval, a decrease in density of the plasma matrix together with a somewhat wider spacing between individual cell particles. This is especially apparent in the subcapsular region and in the adjacent capsule. It persists to various degrees throughout the stages examined in the investigation. It is difficult to attribute a definite pathognomonic importance to this phenomenon, since it may result from an altered reactivity of the tissue to the fixative as a consequence of irradiation or it might be an artifact due to the preparative procedure.

Irradiation-induced nuclear changes are infrequent at the four-day interval. Some structural properties, such as density of nucleoplasm, nuclear boundary, and the number of nuclei per cell, do, however, deviate from the normal condition. A more conspicuous irradiation effect is seen in the cytoplasm at that time in the form of long, lamellated structures which might derive from mitochondria. This interpretation is suggested by observations at the 10-day interval on mitochondria with similar, flattened, multilaminar portions. They closely resemble elements which in another tissue are described as mitochondrial variants.[9]

Several other signs of cytopathology are observed 10 to 14 days after irradiation in epithelial cells. Nuclear changes, observed in both mono- and multinucleated cells, involve the shape of the nucleus, characteristics of the membrane, and the karyoplasm, as well as the site and configuration of nucleoli. They have their correlates in light microscopic observations on flat mounts. The intricate paranuclear complexes, observed once after 24 hours, are more frequently seen at the 10-day interval. Their dense, granular, oval profiles are comparable to structures in deeper lens fibers where they are interpreted as remnants or fragments of nuclei undergoing physiological involution.[10] It is assumed, therefore, that these parts of the agglomerate also derive from nuclear elements. The other more homogenous and less dense formations as well as the very dense, irregular profiles contained in these agglomerates cannot be identified with any of the preexistent cell constituents. It is reasonable to conclude that the dense part of the agglomerates corresponds to the coarse, deeply Feulgen-stained clumps seen in flat mounts. The homogenous, less dense material may correspond to the unstained halos and represent a nonchromatic part of the nucleoplasm.

In other organs, various experimental and pathological conditions, including irradiation effects,[11-14] show mitochondrial changes similar to those observed in lens epithelium. However, they cannot be accurately compared, since, even under normal circumstances, the appearance of mitochondria varies considerably from one organ to the other, although there is a common, basic pattern of organization.

In the two later phases postirradiation effects also involve the endoplasmic reticulum. At first, there is an increase in quantity, although the normal morphology is retained. Subsequently, the profiles become irregular and show a marked increase in the number of attached, dense granules as well as a greater opacity of the intracysternal lumen. Also, the amount of low-density elements is greater, first in circumscribed areas and later throughout the entire cell. Such elements have been identified in lens fibers and are associated with the soluble lens proteins.[15]

In order to designate the small, tortuous, elongated profiles, first observed at the 10-day interval, several characteristics have to be considered in addition to their size and

configuration. They are found only in the vicinity of the lens capsule near interdigitating cell processes and are sometimes contiguous with cell membranes. They may be either arranged at random or aligned in the form of circles or ovals. Whether they originate from tortuous cell membranes, from mitochondria, or the endoplasmic reticulum cannot be ascertained. Their topographical relationship to cell boundaries as well as their organization suggests a provenience from cell membranes. On the other hand, they are similar to structures described in the fetal adrenal gland,[10] where they are interpreted as smooth surfaced endoplasmic reticulum.

Summary

The eyes of young albino rabbits were irradiated with 1,500 r x-rays. The lens epithelium was examined 1, 4, 10, and 14 days after the treatment with the light microscope, in the form of Feulgen-stained flat mounts and with the electron microscope.

Cells affected by ionizing radiation are randomly distributed throughout the epithelium but are more numerous in the equatorial zone. The changes occur in all cell constituents except the Golgi complex and range from slight alterations of the individual components to partial disintegration of the whole cell. The lesions gradually increase with time after irradiation in extent and in the number of structural elements involved. Changes in the organization of nuclei, nucleoli, mitochondria, endoplasmic reticulum, cell membranes, and the fine, low-density components are described in detail and correlated with the data resulting from the Feulgen reaction.

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Evaluation of Ocular Signs and Symptoms in Myasthenia Gravis

N. S. SCHLEZINGER, M.D., and W. A. FAIRFAX, M.D., Philadelphia

Although the neuromuscular disorder known as myasthenia gravis was first described in the seventeenth century,[1] it was not until two centuries later that Erb[2] and Goldflam[3] firmly established the clinical features of this disease. During the next half century, references in the literature dealt mainly with the clinical and pathological aspects of the disease, and this information is well summarized by Keschner and Strauss.[4] Relatively little progress was made in our understanding of the pathogenesis of myasthenia gravis until the advent of the theory of chemical mediation of nerve impulses at synaptic junctions. With the recognition of the importance of acetylcholine in the transmission of the nerve impulse at the motor end plate in striated muscles, there rapidly developed intense interest in both physiologic and pathophysiologic aspects of this mechanism. This interest has been maintained and has led to many significant advances in our knowledge with direct implications as far as the diagnosis and treatment of myasthenia gravis is concerned. These concepts with regard to pathogenesis are described in the contributions to the symposium on myasthenia gravis by Nastuk,[5] Churchill-Davidson and Richardson,[6] and Johns et al.[7] Despite this progress in our understanding of the pathogenesis of myasthenia gravis, the etiology remains obscure. In this regard, the incidence of thymic hyperplasia and thymomas have been provocative observations.

It is doubtful that myasthenia gravis is increasing in frequency. It is more likely that the apparent increase is a consequence of improved recognition of the disease since the discovery of neostigmine as a potent remedy by Walker[8] and its subsequent use as a diagnostic test.[9,10] More recently, edrophonium chloride (Tensilon chloride) administered intravenously has become available as a rapid and reliable alternative diagnostic test.[11,12] The impetus that has been given to the problem of early diagnosis of myasthenia gravis emphasizes the important role of the ophthalmologist. This is readily apparent when one considers that it has long been recognized that the initial symptoms most often appear in the extraocular muscles producing ptosis and variable diplopia.

In recent years, several large series of cases of myasthenia gravis have been reported and the data analyzed from various standpoints. These reports have dealt primarily with patients who have been referred to general hospitals. Even so, there has been a gradually increasing recognition of a form of myasthenia gravis limited to the extraocular muscles.[13,14,15,16] In comparison with such series, our series consists of patients with myasthenia gravis who have been routinely admitted to an eye hospital and subsequently referred to the neurologist. Consequently, our series should be representative of the patients with myasthenia gravis who tend to present themselves to the ophthalmologist.

A survey of the fifty-two patients in our series reveals an age incidence varying from

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From the Department of Neurology, Wills Eye Hospital, and the Department of Neurology, Jefferson Medical College.

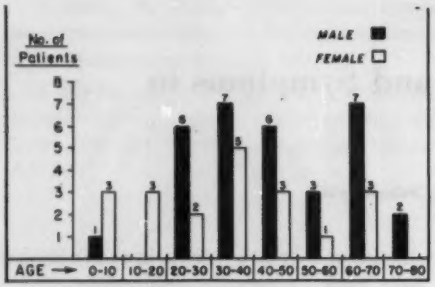

Fig. 1.—Age and sex distribution in Wills Eye Hospital series.

infancy to advanced age (Fig. 1). The youngest had an onset at 18 months, while the oldest had an onset at 75 years of age. The peak incidence in this series occurred in the third to fifth decades with a secondary peak in the seventh decade. It is well to remember that there is a congenital form of myasthenia gravis with onset at birth and which is characterized chiefly by a rather symmetrical bilateral external ophthalmoparesis. An example of such a case (Fig. 2) has been observed in office practice and properly belongs in a series such as this because of referral by an ophthalmologist. It is noteworthy that a ptosis operation was recommended by a number of ophthalmologists throughout the country based upon a presumptive clinical diagnosis of congenital ptosis, and ultimately the operation was performed on one eye before the diagnosis of myasthenia gravis was established.

The sex incidence in this series (Fig. 1) is thirty-two men and twenty women which differs somewhat from the usually described equal sex ratio or slight preponderance of females. Generally, our series shows that the disease tends to affect females predominantly at a younger age while the reverse is true for males, and this is consistent with other reports.[17]

The chief clinical manifestation of myasthenia gravis is fatigability upon sustained effort of affected muscles. The initial symptoms (Fig. 3) most often appear in the form of extraocular muscle pareses producing ptosis and/or variable diplopia. Extraocular muscle involvement may be so severe occasionally as to produce complete external ophthalmoplegia (Fig. 4). Facial muscle involvement, at least to a minimal degree in the orbicularis oculi muscle region, accompanies extraocular muscle pareses in almost every instance. This is responsible for the characteristic myasthenic facies in which there is a tendency towards obliteration of the normal folds, as well as a lack of compensatory furrowing of the forehead in the presence of ptosis. More widespread bulbar muscle involvement affecting the muscles of mastication, palate, pharynx, and larynx may be present initially, or more often constitute a further development during the course of illness. When the bulbar manifestations are conspicuous, it is reasonable to look upon such patients as having a "clinically malignant" form of myasthenia gravis. In some cases, weakness of the muscles of the extremities may represent an early and prominent feature, but only rarely is this unassociated with myasthenic symptoms in the extraocular or bulbar musculature.

In the series of 50 patients available for evaluation during a three-year period (Fig. 5) 16 patients (32%) had ocular myasthenia, while 34 patients (68%) had generalized myasthenia. In the latter group are included 14 patients (28%) with bulbar myasthenic involvement.

In our series, as might be expected, all patients manifested extraocular muscle involvement when first examined, but it is

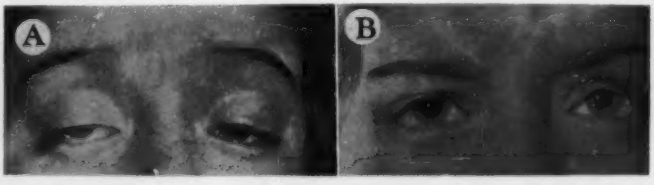

Fig. 2.—A five-year-old girl with congenital myasthenia gravis. *A*. Appearance without medication (prior to ptosis operation). *B*. Appearance with neostigmine medication (after ptosis operation).

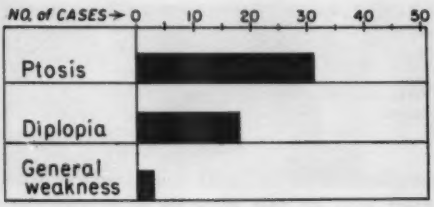

Fig. 3.—Initial symptoms.

interesting to note that in three instances a prior history of generalized fatigue and weakness was obtained which, in each case, had been disregarded. In one woman, these symptoms had been evident since adolescence in regular association with the menses and had antedated ocular myasthenic involvement by 20 years. A survey of our series shows that upon initial examination at the Wills Eye Hospital, 38 patients had involvement limited to the eyes, but further observation of this group over a three-year period (Fig. 6) was limited to 36 cases because two patients failed to return for follow-up visits, and data regarding their first year course is not available. Within three years 20 cases had become generalized, leaving 16 cases that did not become generalized. This latter group is referred to as "ocular," although it must be remembered that slight facial muscle involvement in the region of the eyes is not excluded. It is our belief that this group is similar to those referred to as "ocular" in other reports.[15] Some of these authors have indicated the ocular myasthenia group may be deserving of special consideration in terms of prognosis.[16,18] Our series supports the concept that, after two or three years, it is likely that myasthenia gravis limited to the eyes will remain ocular and therefore constitute relatively little threat to the life of the individual. Of the 20 patients who became generalized during a three-year period of observation, 17 patients manifested this change during the first year, two patients during the second year, and only one patient during the third year.

In our series of patients, the mode of onset has been insidious in the vast majority of cases (Table 1). There apparently was no difference in this regard between the generalized myasthenic group of patients and the ocular myasthenic group. Obviously the data in this respect depends largely upon the observation of the patient and our results

Fig. 4.—A 58-year-old woman with bilateral external ophthalmoplegia. *A*. Without medication. *B*. After 1.5 mg. neostigmine intramuscularly.

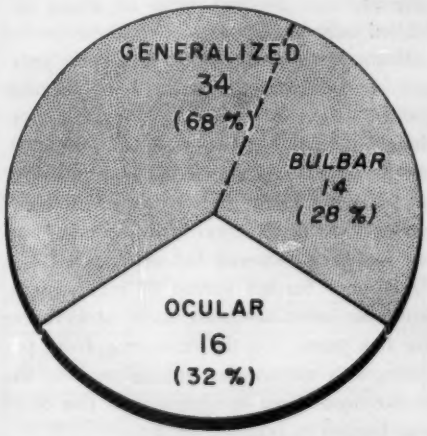

Fig. 5.—Types of myasthenia gravis in Wills Eye Hospital series; a three-year survey in 50 cases.

Duration (years)	No. of Cases	Number Developing Generalized Involvement			
		SLIGHT	MODERATE	MARKED	TOTAL
1	36	10	5	2	17
2	19	0	0	2	2
3	17	1	0	0	1

Fig. 6.—Course of illness in group with initial involvement limited to the extraocular muscles.

are based upon close questioning. The group of patients in whom the onset is indicated as rapid is arbitrarily limited to those in whom the appearance of initial symptoms occurred within a period of less than forty-eight hours.

The initial ocular manifestation involved more than one extraocular muscle in the majority of cases, (Table 2) although the subjective complaint of the patient frequently was limited to the most obvious weakness such as a drooping eyelid, or transient diplopia. It is of interest to note that at the onset of their illness many patients complained of visual difficulty which they described as blurring during ocular concentration and subsequently recognized as diplopia. Objective findings upon initial examination revealed twelve patients with only one eye involved, three of whom exhibited only ptosis. Eleven of these twelve patients were followed at least one year, and seven continued to have involvement limited to one eye only; two of these continued to have only ptosis monocularly, indicating involvement of the levator palpebrae alone. These two patients represent monosymptomatic myasthenia gravis, and one has been followed for nine years (Fig. 7) without further spread of involvement, while the other has been under observation for five years. Of the remaining forty patients with extraocular muscle pareses, the ocular myasthenic involvement in five cases was limited to bilateral ptosis.

It is noteworthy that at the present time much emphasis is being placed upon the

TABLE 1.—*Mode of Onset in Fifty-Two Cases*

	Insidious	Rapid
Generalized	28	8
Ocular	12	4

diagnosis of intracranial aneurysm. A frequent sign of this disorder is extraocular muscle pareses. One patient in our series had been diagnosed as having an intracranial aneurysm, and had been operated upon before referral to the Wills Eye Hospital; carotid artery ligation was performed. Therefore, it is apparent that myasthenia gravis will need to be considered in the differential diagnosis of cerebral aneurysm.

The concurrence of myasthenia gravis and hyperthyroidism has been reported and the question of differential diagnosis commented upon.[19,20,21] The consensus of opinion among neurologists is that myasthenia gravis should be considered a separate disease entity, even in those cases which have hyperthyroidism. Three patients in this series have had such a combination. One male with ocular myasthenia experienced a complete remission with the control of hyperthyroidism that has persisted for seven years. One woman was not benefited by the control of hyperthyroidism and, because of rapidly progressive bulbar muscle involvement, was subjected to thymectomy with death occurring postoperatively. Another woman is currently responding favorably to methimazole (Tapazole) therapy.

Pregnancy has been observed to have a variable effect upon myasthenia gravis.[22,23] In our series, two patients are known to have had pregnancies following the development of myasthenia gravis. One patient with ocular myasthenia manifested no change

TABLE 2.—*Extent of Extraocular Muscle Involvement*

	Monocular	Binocular
Ptosis Only	3	5
Multiple E.O.M. Pareses	9	35

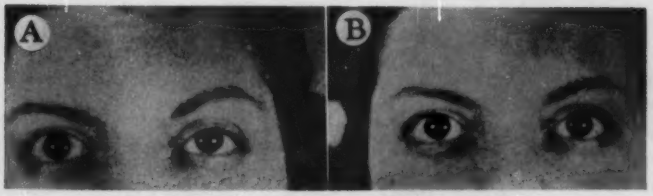

Fig. 7.—A 30-year-old woman with monosymptomatic myasthenia gravis in the form of slight left ptosis. *A*. Without medication. *B*. After 1.5 mg. neostigmine intramuscularly.

during and after each of two pregnancies which resulted in the birth of a deformed child that died, and then a normal child. The other patient with generalized myasthenia improved throughout pregnancy, but had a partial relapse subsequent to the birth of an infant, which died as a result of neonatal myasthenia that was not recognized by the obstetrician.

The treatment for the vast majority of patients with myasthenia gravis in our series (Fig. 8) has consisted of either neostigmine or Mestinon, usually combined with ephedrine unless contraindicated. This therapy has proved to be satisfactory in controlling symptoms of the generalized and bulbar groups except when the bulbar manifestations were so marked as to constitute a threat to life. In such instances, thymectomy was performed and seemed to be effective in the younger age group that survived the operation. Most of the operative deaths occurred in the period before adequate preoperative and postoperative care was arranged on the basis of close collaboration between the thoracic surgeon and neurologist. This was true in three of four deaths in our series that occurred in the immediate postoperative period.

A survey of the ocular myasthenic patients in our series demonstrates that the extraocular muscle involvement in many instances has been relatively refractory to neostigmine or Mestinon in terms of only partial subsidence with considerable residual involvement. This results in a variable degree of persistent disability both from cosmetic and occupational standpoints. A noteworthy observation has been that corticotropin (ACTH) therapy administered in a total dosage of 600 mg., divided into single doses of 20 mg. every six hours, is highly effective ordinarily in reducing or eliminating the extraocular muscle pareses. This beneficial result is observed, when it occurs, as a "rebound" phenomenon within forty-eight to seventy-two hours after the termination of corticotropin therapy. The duration of the partial or complete remission has varied from three to as long as twelve months. Relapse is to be expected, but in the ocular myasthenic group, it often may be delayed and may be relatively mild. When necessary corticotropin therapy has been repeated; the interval is usually not less than six months. Although corticotropin therapy understandably has been largely rejected for the treatment of myasthenia gravis which is generalized, it would seem reasonable to continue its use in the treatment of selected patients with ocular myasthenia in whom the disability affecting the eyes cannot otherwise be adequately controlled.

Summary

A series of 52 patients with myasthenia gravis have been observed at the Wills Eye Hospital within the past 16 years. This series is considered to be representative of the patients with myasthenia gravis who would be apt to present themselves to the ophthalmologist. The entire series has been surveyed with regard to age and sex in-

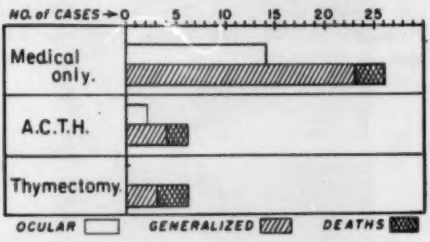

Fig. 8.—Modes of therapy and results obtained.

cidence, mode of onset, course of illness, and treatment.

Within a three-year period, 16 patients were observed to have myasthenic involvement which remained limited to the eyes. This "ocular" group appears to have a relatively good prognosis in terms of life expectancy.

The treatment of ocular myasthenia may include corticotropin therapy in selected instances when extraocular muscle pareses are relatively refractory to neostigmine or Mestinon medication.

255 S. 17th St. (3).

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Agar Microelectrophoresis at High Tension of Soluble Lens Proteins in Cataract

J. FRANCOIS, M.D., and M. RABAEY, M.D., Ghent, Belgium

Various authors have demonstrated that the quantity of soluble lens proteins diminishes in the course of development of a cataract, whereas the quantity of albumin increases, particularly in human specimens (Jess, 1913; Hektoen and Schulhof, 1924; Lavagna, 1926; Hertel, 1933; Jayle, Derrien, and Ourgaud, 1944, and Dische and Zil, 1951).

No exact data are available as yet, however, as to the changes which the various soluble proteins are subject to in the course of opacification of the lens, and there is no certainty yet as to the fraction which is first and most markedly affected during this process.

Investigations by Smelser and von Sallmann (1949), into lenses from mice suffering from hereditary cataract, and those by Lewis and Moses (1950), into alloxan-induced cataract in rabbits, have been made with the aid of free electrophoresis according to Tiselius.

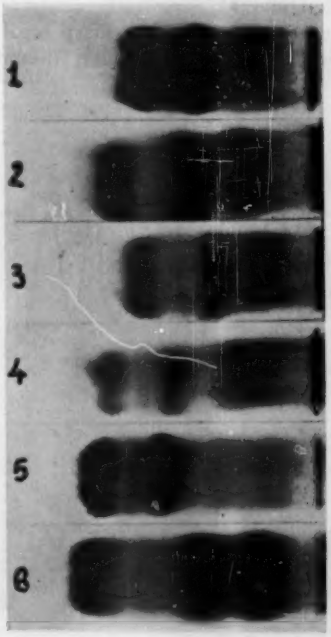
1. Our investigations into the pathogenesis of galactose cataract (1954) have shown that the wet weight of the lens is increased by about 50% as compared with the normal lens. In this respect it must be pointed out that, in our series, the lenticular opacities occurred early and showed rapid development.

The ratio of the various soluble protein fractions, which were markedly reduced in quantity in galactose cataract, was studied by means of paper electrophoresis.

In normal rats, the paper pherograms showed four to five components which may be divided into three fractions. Fraction I corresponds with α-crystallin. In Fraction II we distinguished a more rapid and a slower component, the respective percentages being 15% and 85%. In Fraction III, which prevails in the nucleus, two components are likewise sometimes distinguishable.

In cataractous lenses the diminution of soluble proteins does not affect a single fraction, but involves the three fractions simultaneously and almost to the same extent. In fact, and unless the opacity has become too extensive, the ratio between the three fractions in cataractous lenses is the same as that in normal lenses.

Fig. 1.—Paper electrophoresis of soluble proteins of the normal lens in rat; Nos. 1 to 4=total lens; No. 5=cortex; No. 6=nucleus.



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From the Ophthalmological Clinic of the University of Ghent.

When opacification is very marked the proportion is slightly modified in favor of Fraction I and at the cost of Fraction III. This fact is particularly evident in the case of chiefly nuclear opacities, and it is this circumstance which affords an explanation of the shift. If the soluble proteins have disappeared principally in the nuclear part (nuclear cataract), then the soluble proteins of the cortical part, where normally Fraction I is larger and Fraction III smaller, will predominate in the total lenticular extract.

With regard to the subdivision of Fraction II into two components, it can be said that the proportion of these components in the cataractous lens differs from that in the normal lens.

Although there is no difference between normal and cataractous lens in the quantitative proportions between the soluble proteins, it can nonetheless be observed that important qualitative differences exist in the pherograms; it is these differences which make it possible immediately to differentiate between a cataractous and a normal lens (Figs. 1 and 3).

It can be seen that the sharp separation between the various fractions disappears, the fractions widen, extend and become indistinct. In the diagrams the peaks are largely curved (Figs. 2 and 4).

Among other things, it is seen that a great many diagrams of cataractous lenses show more peaks than those of normal lenses. It is not uncommon to find seven to eight different peaks. The subdivision into several components concerns not only Fractions II and III but also Fraction I (α-crystallin). The significance of this "decomposition" into numerous components is still obscure, particularly since it is known that the protein composition of the mammalian lens is complex and comprises three to four soluble fractions.

It may well be that, in the course of cataract formation, certain protein fractions develop a new division.

2. We have also examined rabbit lenses first exposed to *x-rays* (1500-2500 r), and then developing cataract after a latent period of two to four months. These investigations were particularly interesting as we could compare cataractous and normal lenses from the same animal. We compared whenever possible the lenticular weight, the total protein value, the percentage of soluble pro-

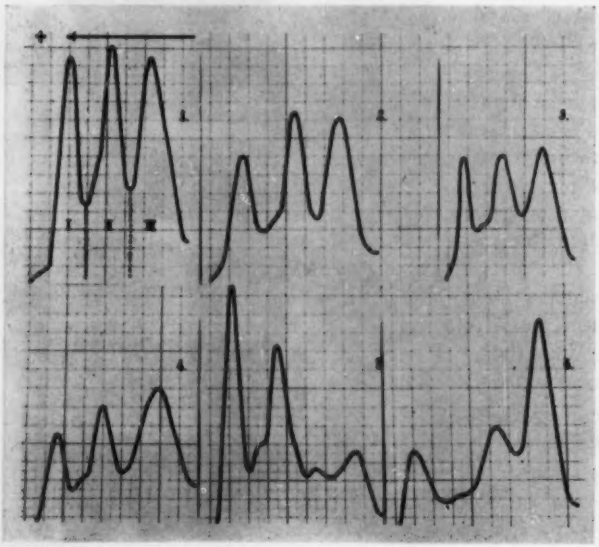


Fig. 2. — Diagram of the pherograms of Fig. 1.

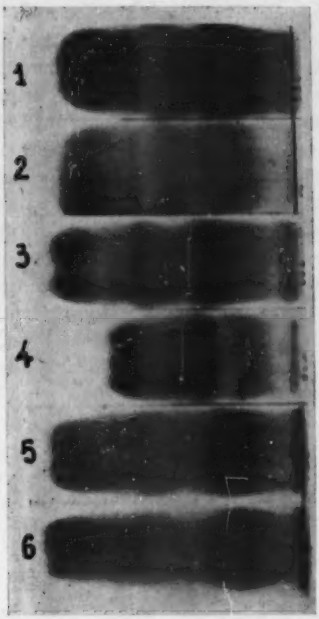


Fig. 3.—Paper electrophoresis of soluble proteins of the cataractous lens in rat; No. 1 to 6= total lens; the lens No. 1 showed only slight diffuse opacities.

teins, and the proportion of the various fractions after paper electrophoresis.

(*a*) After irradiation, but during the latent period, before the occurrence of the first clinical signs of lenticular opacification, no change in the protein composition of the lens was demonstrable.

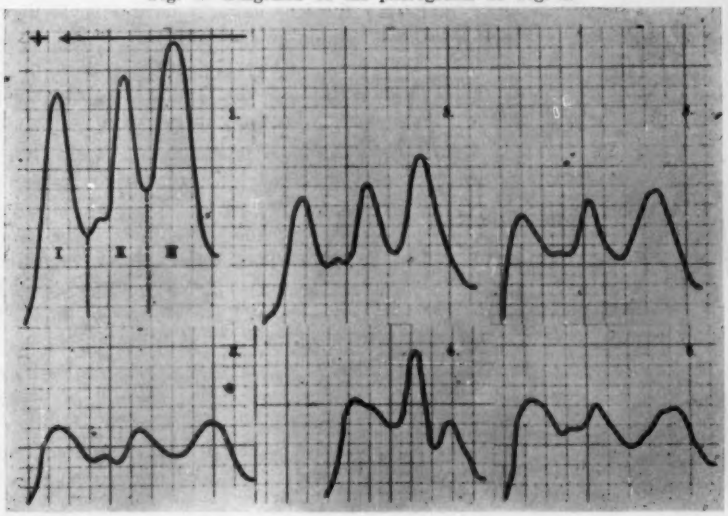
(*b*) In rabbits exposed to 2000-2500 r, the weight of the cataractous lens was always above that of the normal lens. The swelling was sometimes very marked, and the increase in lenticular weight was sometimes over 30% in extreme cases.

In view of this more or less marked increase in volume, the protein concentration in the cataractous lens might be expected to be less than that in the normal lens. This is in fact the case, but the difference in the majority of cases was more marked than could be explained on the basis of lenticular swelling exclusively. In all the cases examined, the total protein value of the cataractous lens was considerably lower than that of the normal lens. The total proteins in the cataractous lens, on the other hand, consist largely of insoluble albumin.

It can be concluded, therefore, that cataract formation following x-ray treatment in rabbits is associated not only with an increase in albumin but also with the disappearance of part of the proteins.

In rabbits exposed to no more than 1500 r, cataract develops considerably slower

Fig. 4.—Diagrams of the pherograms of Fig. 2.



and only after a latent period of three to seven months, or longer. In these cases the lenticular volume is not increased. On the contrary, it decreases in such a manner as to suggest lenticular sclerosis.

(*c*) Paper electrophoresis reveals three protein fractions in normal rabbits; the average percentages being 24.1% for Fraction I (α-crystallin), 51.6% for Fraction II, and 24.3% for Fraction III.

We have given special attention to modifications in the proportion between these three fractions in the case of cataract produced by x-rays. Unfortunately, we are unable to present averages; the results obtained were so variable that an average figure would have no significance whatever. On the other hand, it is sometimes impossible to distinguish in diagrams of cataractous lenses the three fractions of the normal lens.

In certain cases Fraction I (α-crystallin) is the fraction best preserved. In other cases it is the slow fractions which constitute the larger part of the proteins. In the extreme cases where only a very small quantity of soluble proteins remains, these are seen to migrate to the place where Fraction III is normally encountered.

Among other things, the pherograms showed changes similar to those seen in galactose cataract in rats (Figs. 5 and 6). The fractions were less sharply separated and the peaks less high; the fractions themselves were more extensive and more blurred. There was also considerable adsorption onto the filter paper during migration. Many of the diagrams showed numerous peaks. This decomposition into multiple constituents was sometimes so marked, and always so variable, as to make it difficult or even impossible to distinguish the three normal lens fractions.

Our investigations into human cataract have shown that the behavior of lenticular proteins was entirely different from that observed in animals. The majority of human cataracts examined by us were senile cataracts.

Let us first consider the data to be found in the literature. In a number of cases, Croisy (1952) found an increase in the most rapid fraction, whereas other cases showed decreases, and still other cases failed to show any difference between cataractous and normal lenses. Stemmerman (1953), and also Frezotti and Scagnetti (1957), observed absence or diminution of one of the two fractions which they obtained by paper electrophoresis at pH 6.6.

In general, we have also seen differences between the pherograms of cataractous human lenses and normal human lenses, at least after very careful centrifugation of the lens extracts until a perfectly clear solution was obtained.

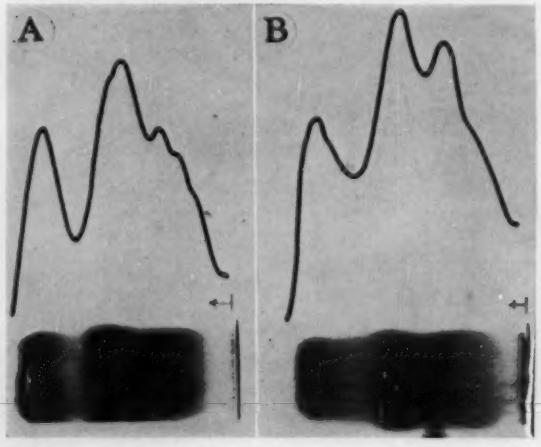


Fig. 5.—Paper electrophoresis of soluble lens proteins in rabbit after irradiation. A=normal lens, B=irradiated lens (2,500 r).

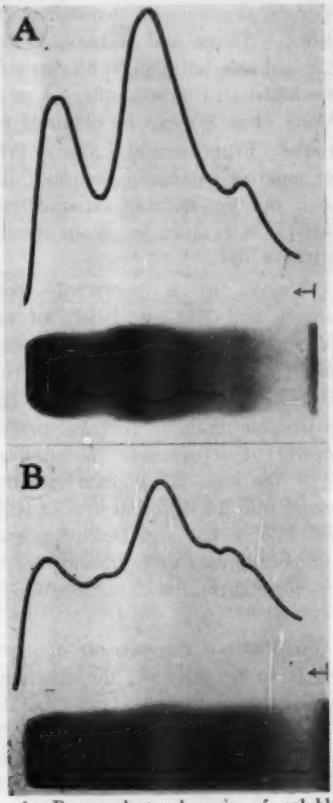


Fig. 6.—Paper electrophoresis of soluble lens proteins in rabbit after irradiation. A=normal lens, B=irradiated lens (200 r+500 r).

The pherograms of cataractous lenses showed that the proteins migrate a little farther on filter paper than those of normal lenses. The zone over which the proteins are distributed is at the same time smaller because, and this is an important observation, the slow constituents in particular were diminished (Figs. 7 and 8).

As in the cataract cases the quantity of soluble proteins was reduced—it was hardly possible to speak of an increase in the more rapid constituents. These, too, migrated very close together as a rule, and separation of fractions was often hardly discernible.

In the majority of cataractous lenses examined, the quantity of proteins was nearly the same as that in normal lenses.

The average wet weight of 14 normal lenses from subjects aged over 40 was 221.7 mg., whereas that of 17 lenses with senile cataract amounted to 194.6 mg. The cataractous lenses therefore weighed a little less than did the normal lenses; the difference, however, was minimal.

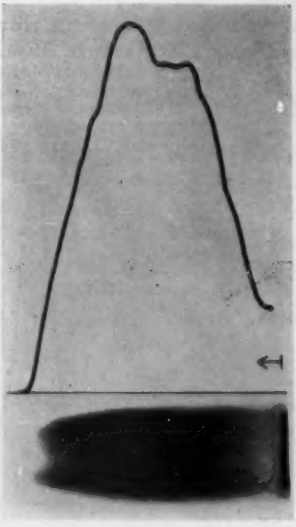
The average level of protein nitrogen per lens was in the same manner a little larger in normal lenses (10.66 mg.) than in cataractous lenses (9.88 mg.). The quantity of protein nitrogen per 100 mg. fresh lenticular substance, however, was the same in normal and in cataractous lenses (4.93 in the former and 5.05 mg. in the latter).

Only in some cases of far advanced cataract was a certain quantity of protein eliminated from the lens.

However, that which should be chiefly taken into account in senile cataract is the transformation of soluble proteins into insoluble albumin.

In normal lenses at a given age, the percentages of soluble proteins show no great variations. There is a slight decrease, however, with increasing age (up to about 70%). The results with regard to cataractous lenses are considerably more irregular.

Fig. 7.—Paper electrophoresis of soluble proteins of the normal lens in a man aged 50.



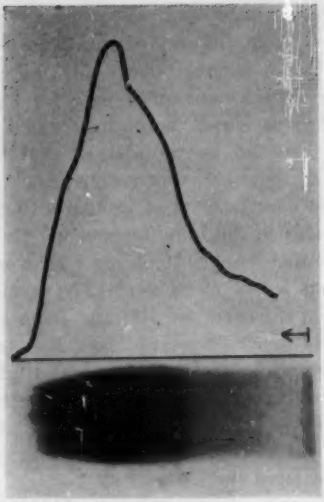


Fig. 8.—Paper electrophoresis of soluble proteins of the cataractous lens in a man aged 72.

In some cases, in which the opacification is still incipient, the percentage of soluble proteins is the same as that in normal lenses of the same age group. In the majority of cases, however, the level of soluble proteins per 100 mg. of lenticular substance is more or less markedly reduced. In certain cases the percentage found does not exceed 20%-30%. All values between this level and the normal level may be encountered.

Agar microelectrophoresis at normal temperature makes it possible to obtain a sharper separation of lenticular protein fractions (Wieme and Rabaey, 1956), not only in animals but also in human subjects.

In children, for example, at least 10 fractions (Fig. 9) can be obtained by this technique (François and Rabaey, 1957).

For aged or cataractous lenses this technique is not yet entirely satisfactory, particularly if it is used to obtain comparable quantitative data.

3. Thanks to a perfected technique (François and Rabaey, 1958) of electrophoresis at low temperature (4 C) and high tension (potential gradient of electric field 20-40 volts per cm.), however, it has become possible again to increase perceptibly the power of separation. The soluble proteins of the lens in children can thus be separated into 14 different fractions (Figs. 10 and 11); it has also become possible to submit pherograms of aged and cataractous lenses to quantitative fractionation (Fig. 12).

If quantitative comparison of data obtained is to be possible, the fractions obtained in the pherograms must be divided into groups of different mobility (Table). The α-crystallin fraction has a relative mobility of about 0.90 * and generally is ex-

* The mobility values given are relative to those of human serum albumin, the mobility of which is considered to equal +1. The zero point is indicated by dextran.

Fig. 9.—Agar microelectrophoresis of soluble lens proteins in a child aged 14 months. Temperature: 25 C; potential gradient of electric field 10 volts per cm. during 25 minutes.

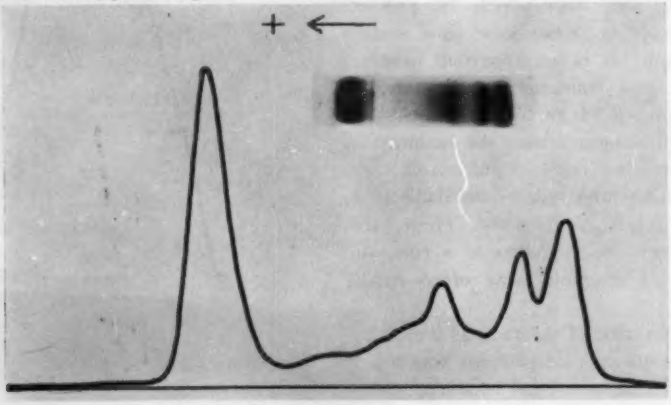


TABLE.—*Zones of Mobility of the Various Fractions and Groups of Fractions*

	Mobility Per Fraction	Mobility Per Group of Fractions
Rapid fractions R	>1.1	>1.1
α-Crystallin	0.75←→1.1	0.75←→1.1
Fraction M1	0.63←→0.73	
Fraction M2	0.53←→0.63	0.44←→0.73
Fraction M3	0.44←→0.53	
Fraction V1	0.34←→0.44	0.25←→0.44
Fraction V2	0.25←→0.34	
Fraction T	0.17←→0.25	0.17←→0.25
Fraction S	0.07←→0.17	0.07←→0.17

tensive when aged lenses are concerned. The rapid fractions are those whose relative mobility exceeds 1.1. The three groups M_1, M_2, and M_3, which are also rather extensive, have a mobility which is less than that of α-crystallin. They are followed by groups V_1 and V_2, which are readily separated in children and adolescents, but offer greater difficulty in aged subjects. Next comes peak T, which is seen at a very marked band, at the slow fractions S_1 and S_2.

It is a striking fact that part of the α-crystallin from aged lenses shows little or no migration in agar even after prolonged centrifugation of the lens extracts. This protein remains on or near the starting line. We have designated this a semisoluble α-crystallin, which owes its appearance to polymerization which progresses with increasing age.

Some 40 cataractous lenses were examined by agar electrophoresis at high tension (Fig. 13), with quantitative determination of the importance of the various fractions in 23.

In the majority of cataractous lenses the percentage of soluble proteins was lower than that in normal lenses of the same age, as a result of the transformation of part of the soluble proteins into insoluble albumin. This does not hold true, however, for *all* such lenses as show opacities. The pherograms and the quantitative data show that, in these cases, there is no difference between the cataractous lens and the normal lens of identical age.

Reviewing the quantitative data we find that, in principle, all fractions may be diminished in cataractous lenses. This diminution is most marked, however, and most regular in the fractions of low mobility (groups S, T, and, to a certain extent, V1 and V2). The average fractions (M) show the least modifications; it is only in exceptional cases where the percentage of soluble proteins is minimal that these are also diminished. In nearly all cataractous lenses the total quantity of α-crystallin (soluble and semisoluble) is smaller than that in normal lenses. In the majority of cataractous lenses the quantity of semisoluble α-crystallin is also smaller than that in normal lenses.

During insolubilization of proteins at the start of cataract formation (a process which largely takes place at the cost of the fractions of low mobility), semisoluble α-crystallin, which is already markedly polymerized, is precipitated at the same time.

It must be pointed out that the phenomena concerning proteins early in the course of senile cataract formation are to a certain extent similar to those seen in the course of the process of aging of the lens, the difference being that they are accelerated. In the normal lens there is also a diminution of the quantity of fractions of low mobility as the subject advances in age; this diminution, however, is more intensive in the cataractous lens.

In the final stage of cataract formation (total cataract), other manifestations can be observed. Not only is there a marked decrease in the quantity of soluble proteins, but there is also a decrease in the

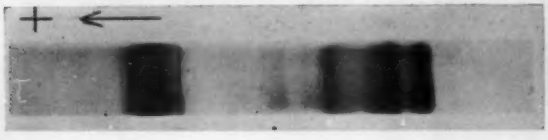


Fig. 10.—Agar microelectrophoresis of soluble lens proteins in a neonate. Temperature 4 C.; potential gradient of electric field 20 volts per cm. during 25 minutes.

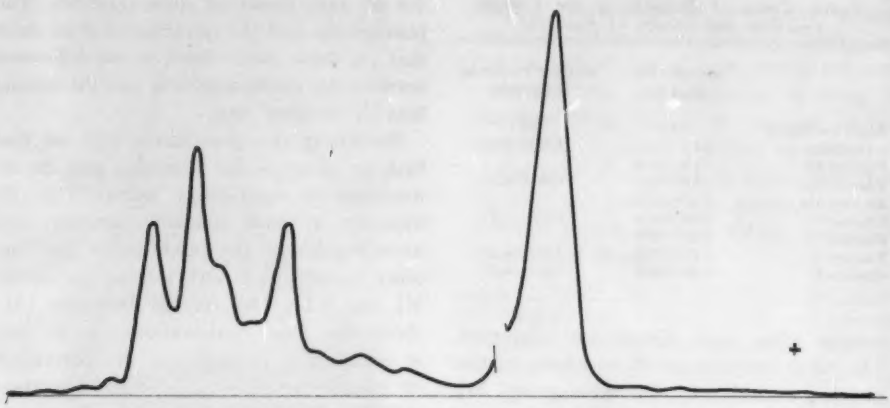


Fig. 11.—Diagram of the pherogram of Figure 10.

total protein level per 100 mg. lenticular substance. The pherograms meanwhile show that α-crystallin is always present in a relatively high concentration whereas all other fractions (including the M fractions) are markedly diminished. Only fraction T is still recognizable by a well-circumscribed band. In group V, too, a marked band is sometimes seen to occur.

It may be concluded that, in the stage of complete cataract, a nonspecific progress begins which renders lenticular proteins insoluble and removes them from the lens. Only α-crystallin constitutes an exception to this rule; it presents the major portion of the soluble proteins still present in the lens.

Summary

Cataract is due to opacification of the lenticular tissue associated with an irregular modification on the index of refraction, the increase of which is chiefly determined by the protein concentration.

The total protein concentration in a cataractous lens hardly differs from that in a normal lens of the same age. However, whereas the percentage of soluble proteins

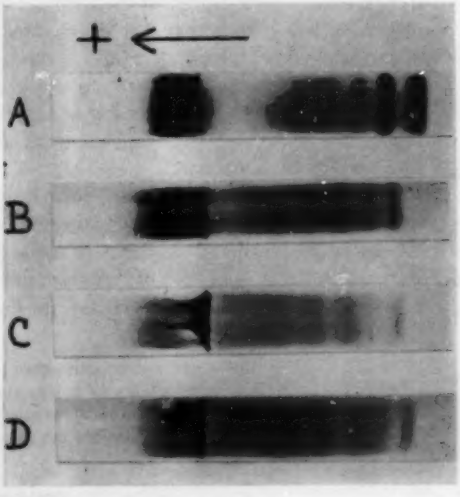


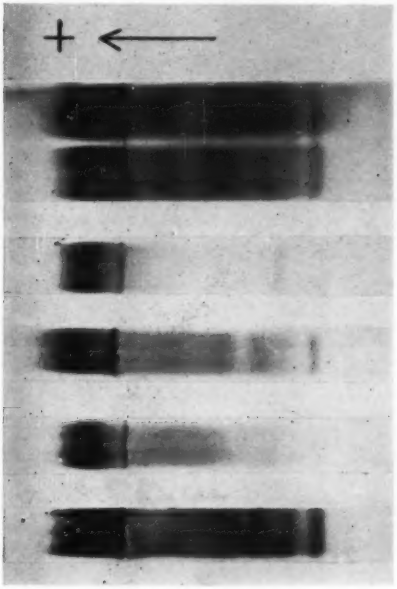
Fig. 12.—Agar microelectrophoresis of soluble proteins of human lenses of different age. A=4 years; B=35 years; C=58 years; D=70 years.

in a normal lens of certain age remains constant within certain limits, this percentage shows marked variations in the cataractous lens. It can be stated, in general, that the percentage is smaller as the cataract formation is more advanced. The old methods of fractionation are meanwhile incapable of providing information as to which protein fraction is the first or the most markedly affected.

Paper electrophoresis separates the various fractions of the proteins in the adult lens in a very rudimentary fashion, and affords no opportunity to demonstrate with certainty a difference between the normal and the cataractous lens.

A new technique of agar microelectrophoresis at high tension, however, has enabled us to obtain very advanced fractionation (at the same time very rapid), both in normal and in cataractous lenses. By this technique we have been able to separate lenticular proteins into numerous fractions (9) or several groups of fractions (6).

Fig. 13.—Agar microelectrophoresis of soluble proteins of human cataractous lenses.



In principle, all fractions may be diminished in cataractous lenses; this diminution, however, is most marked and most regular in the fractions of low mobility, at the cost of which the proteins are rendered insoluble at the beginning of cataract formation.

The protein changes seen at the beginning of senile cataract formation are analogous to those observed in the course of lenticular aging, the difference being that they are accelerated.

In the final stage of lenticular opacification, the total protein concentration decreases due to removal from the lens. The only exception to this rule is found in α-crystallin.

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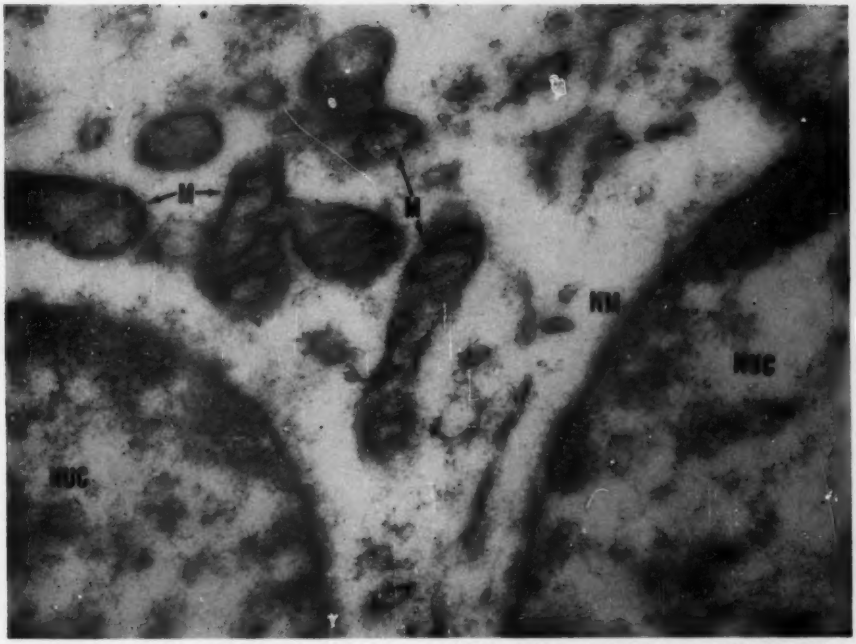


Fig. 2.—Thin section of an osmium tetroxide-fixed, methacrylate-embedded epithelial cell. Portions of the nucleus (*NUC*) with the nuclear membrane (*NM*) are seen. In the adjacent cytoplasm are mitochondria (*M*) showing characteristic double membranes. Magnification × 54,000.

The figures on this page and page 1002 are for the article on pp. 931 to 934 by Fine et al.

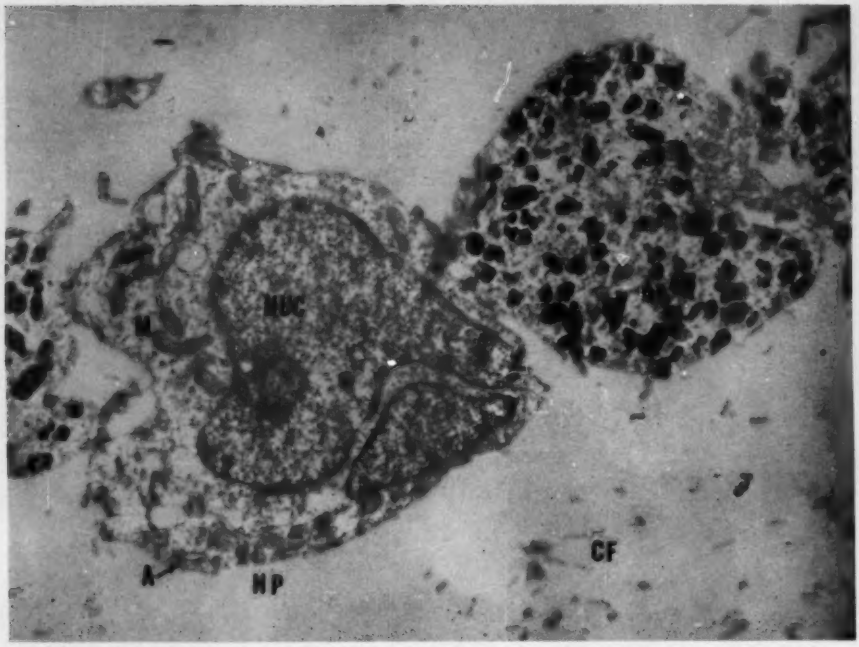


Fig. 3.—Thin section of human iris showing pigmented and nonpigmented stromal cells. Within the nonpigmented cell is its nucleus (*NUC*), the cytoplasm with its mitochondria (*M*), and portions of α-cytomembranes (*A*). On either side of this cell are portions of pigmented cell cytoplasm, containing pigment granules. Collagen fibrils (*CF*) are seen extracellularly. Magnification × 13,800.

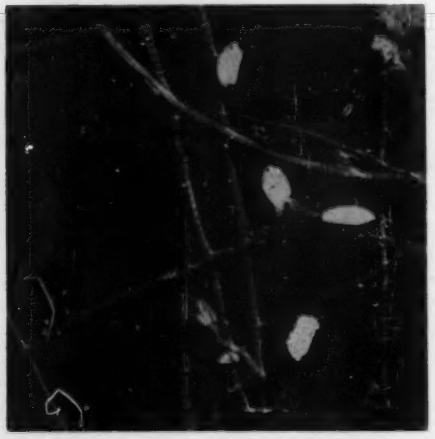
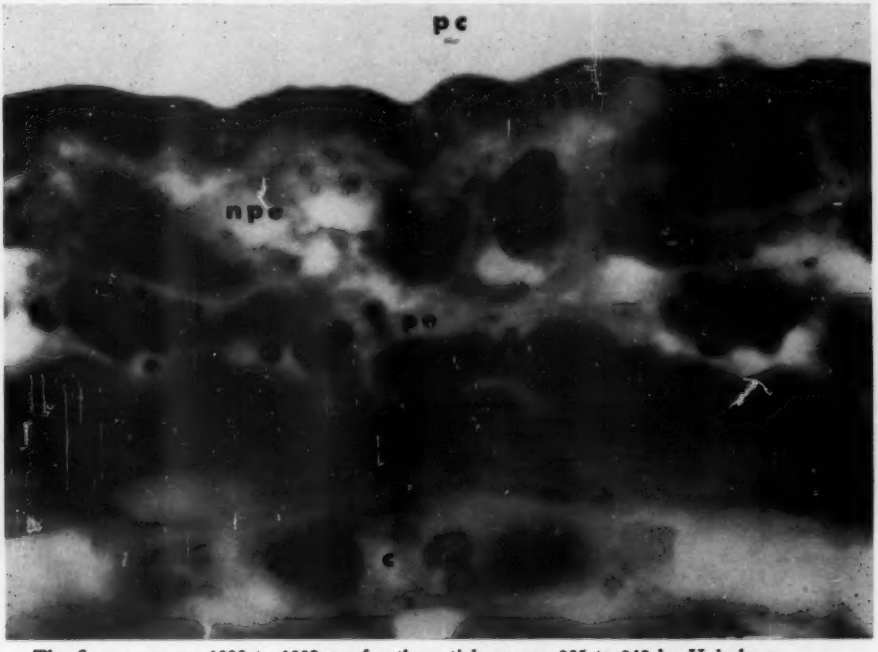


Fig. 5.—Pigment granules and collagen fibrils isolated by centrifugation from homogenized formalin-fixed human iris stroma then shadow-cast with uranium. In this negative print the pigment granules appear white and their shadows black, while in Figure 3, a positive print, the granules appear black; Magnification × 13,800.

Fig. 1.—Light micrograph of a ciliary process, human eye. Yamashita, Cibis, and Becker, 1959. × 800.

Fig. 2.—Higher magnification of a part of Figure 1. *c* indicates capillary; *elm*, external limiting membrane; *pe*, pigment epithelium; *npe*, nonpigmented epithelium; *ilm*, internal limiting membrane; *pc*, posterior chamber. × 1,600.



The figures on pp. 1003 to 1032 are for the article on pp. 935 to 948 by Holmberg.

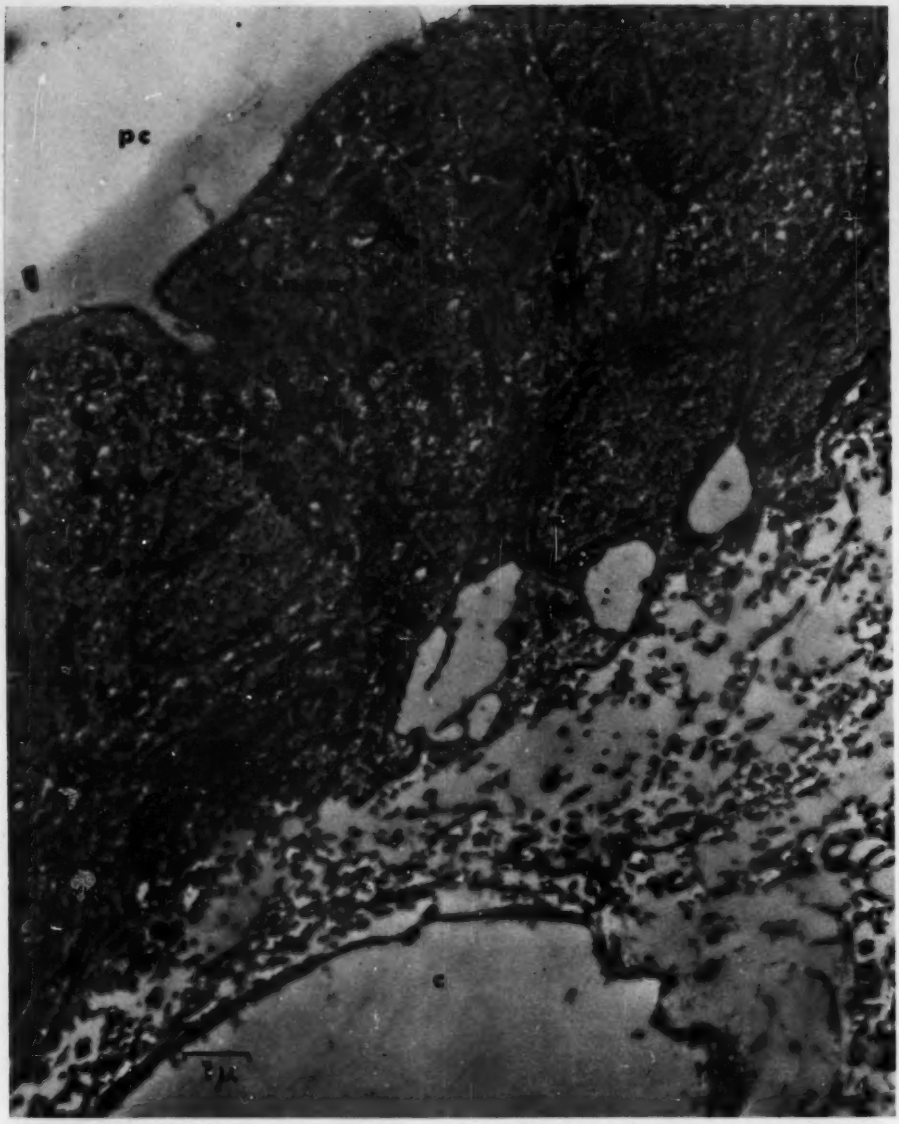


Fig. 3.—Survey of a ciliary process, electron micrograph. *c* indicates capillary; *s*, ciliary stroma; *pe*, pigment epithelium with nucleus (n_1); *npe*, nonpigmented epithelium with nuclei (*n*), *pc*, posterior chamber. Rabbit eye; × 10,000.

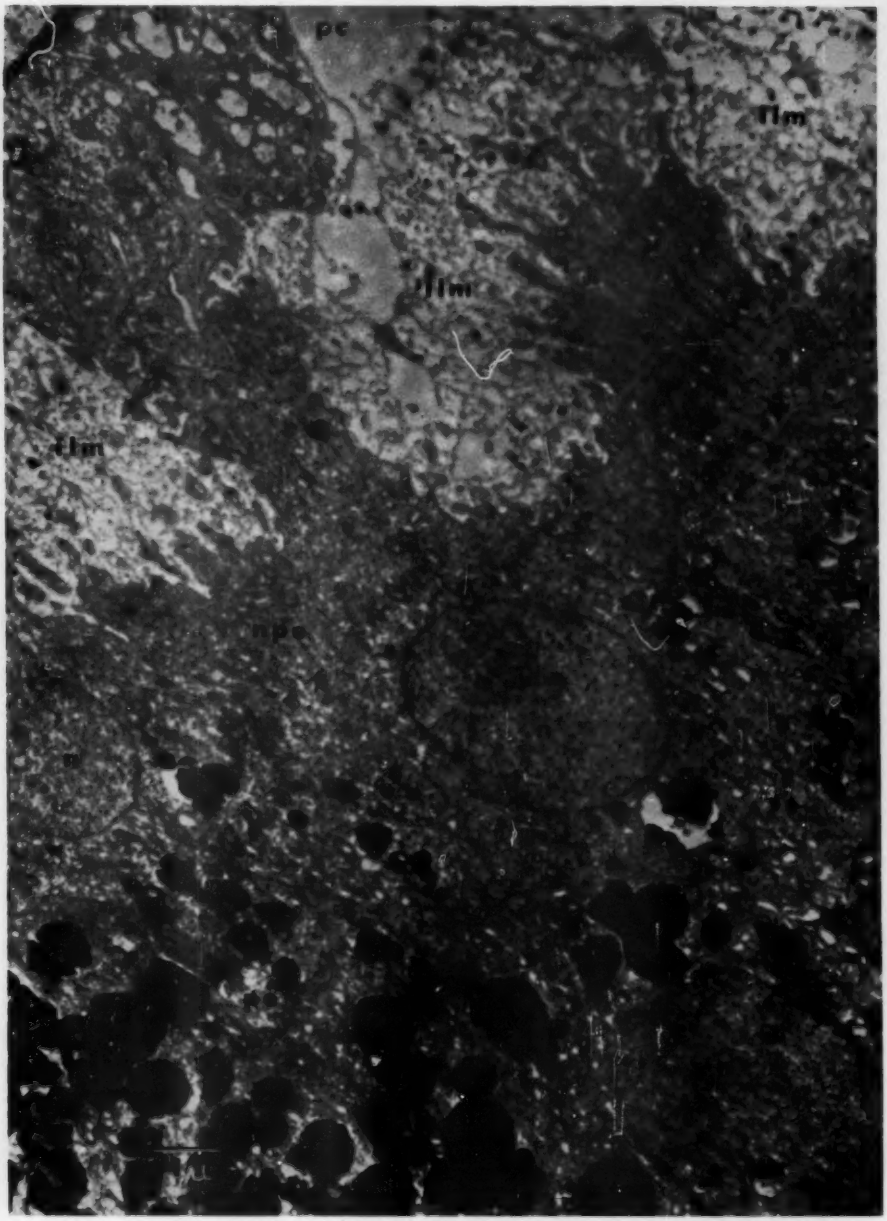


Fig. 4.—Apical part of the pigmented epithelium (*pe*), the nonpigmented epithelium (*npe*), internal limiting membrane (*ilm*), and posterior chamber (*pc*). *n* indicates nuclei. Human eye; × 10,000.

Fig. 5.—β-Cytomembranes (*β-cm*) in the nonpigmented epithelium. *pc* indicates posterior chamber; *m*, mitochondria. Rabbit eye; × 50,100. Reproduced from Holmberg, A.: Thesis, Stockholm, 1957.

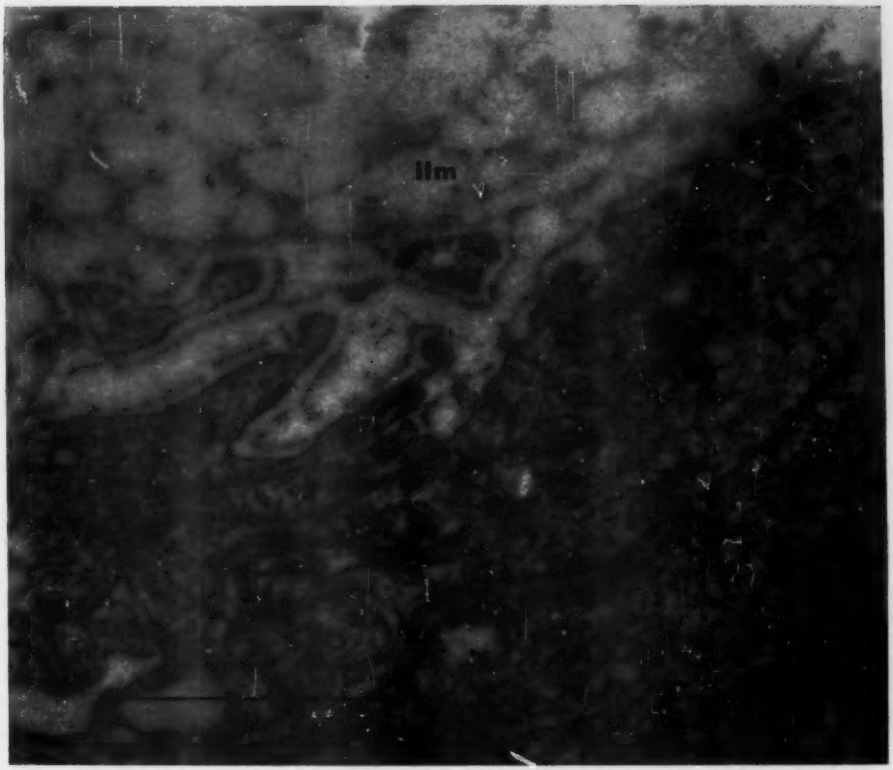


Fig. 6.—β-Cytomembranes (β-*cm*) in the nonpigmented epithelium. *ilm* indicates internal limiting membrane. Human eye; × 42,200.

Fig. 7.—Highly folded cell border (*cb*) in the nonpigmented epithelium. *n* indicates nucleus; *m*, mitochondria; *pc*, posterior chamber. The arrows indicate the internal limiting membrane. Rabbit eye: × 57,600. Reproduced from Holmberg, A.: Thesis, Stockholm, 1957

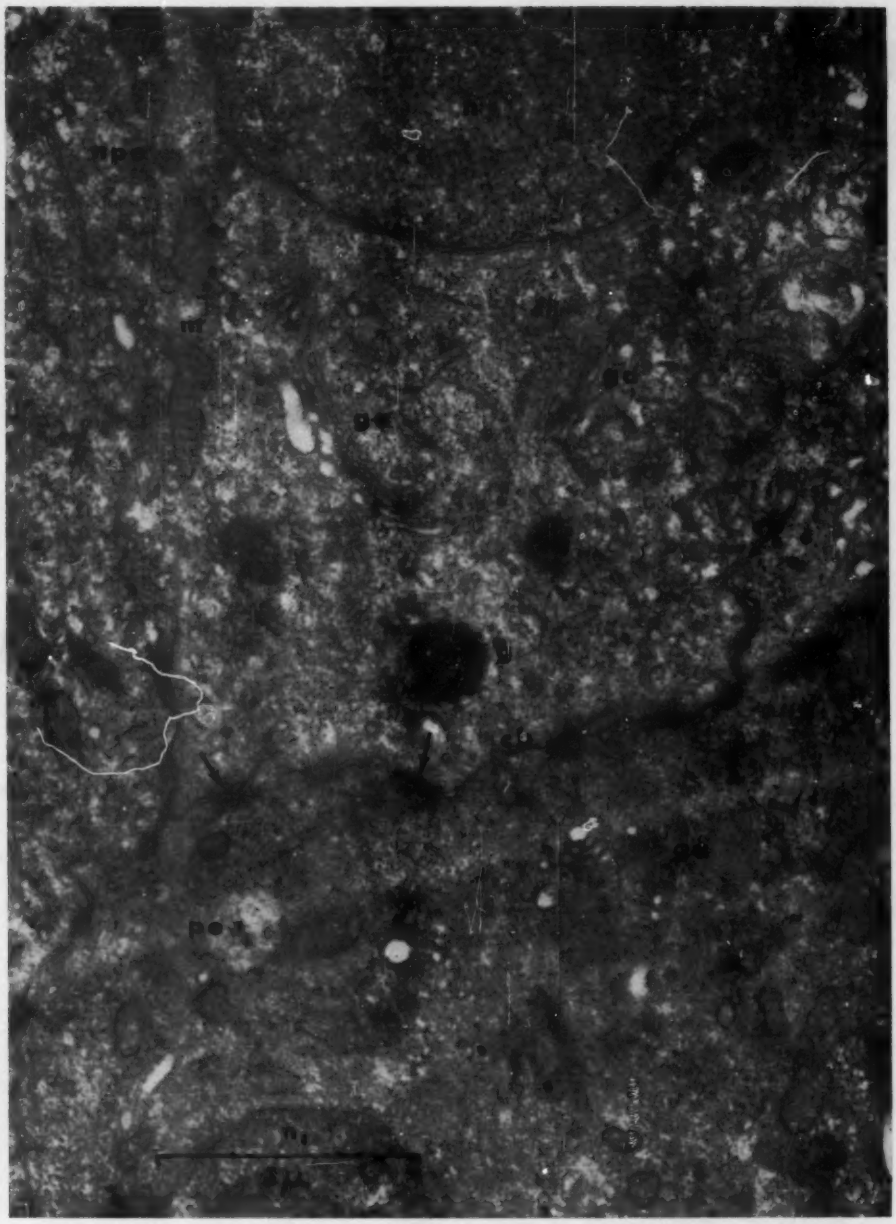


Fig. 8.—Apical part of the pigment epithelium (*pe*) and basal part of nonpigmented epithelium (*npe*). *n* and n_1 indicate nuclei; *m*, mitochondria; *ga*, Golgi apparatus; *g*, granula; *cb*, cell border. The arrows indicate the terminal bars. Note the difference in density of the two epithelia. Rabbit eye; × 41,000. Reproduced from Holmberg, A.: Thesis, Stockholm, 1957.

Fig. 9.—α-Cytomembranes (arrows) in the nonpigmented epithelium. *cb* indicates cell border; *n*, nucleus. Rabbit eye; × 47,100.

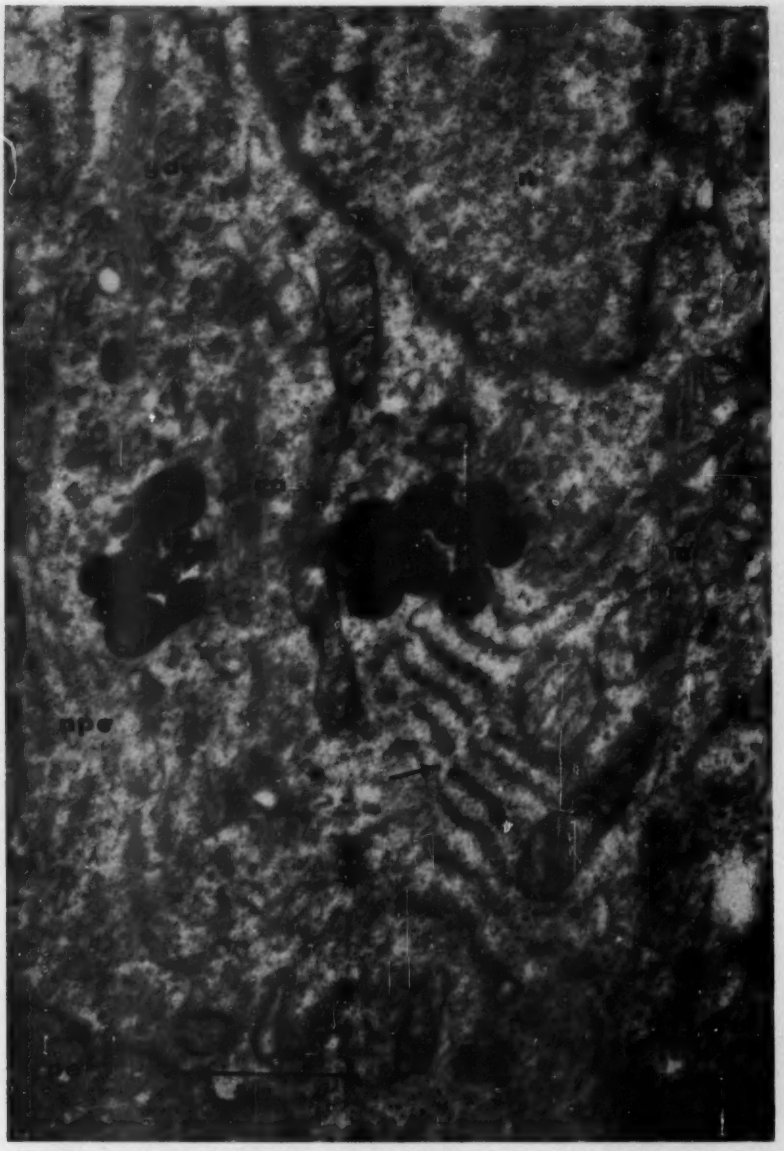


Fig. 10.—Basal part of the nonpigmented epithelium (*npe*) and a small part of the pigment epithelium (*pe*). The arrow indicates a group of α-cytomembranes. *m* indicates mitochondria; *ga*, Golgi apparatus; *n*, nucleus. Two aggregates of osmiophilic granules are seen in the central part of the picture. Human eye; × 30,000.

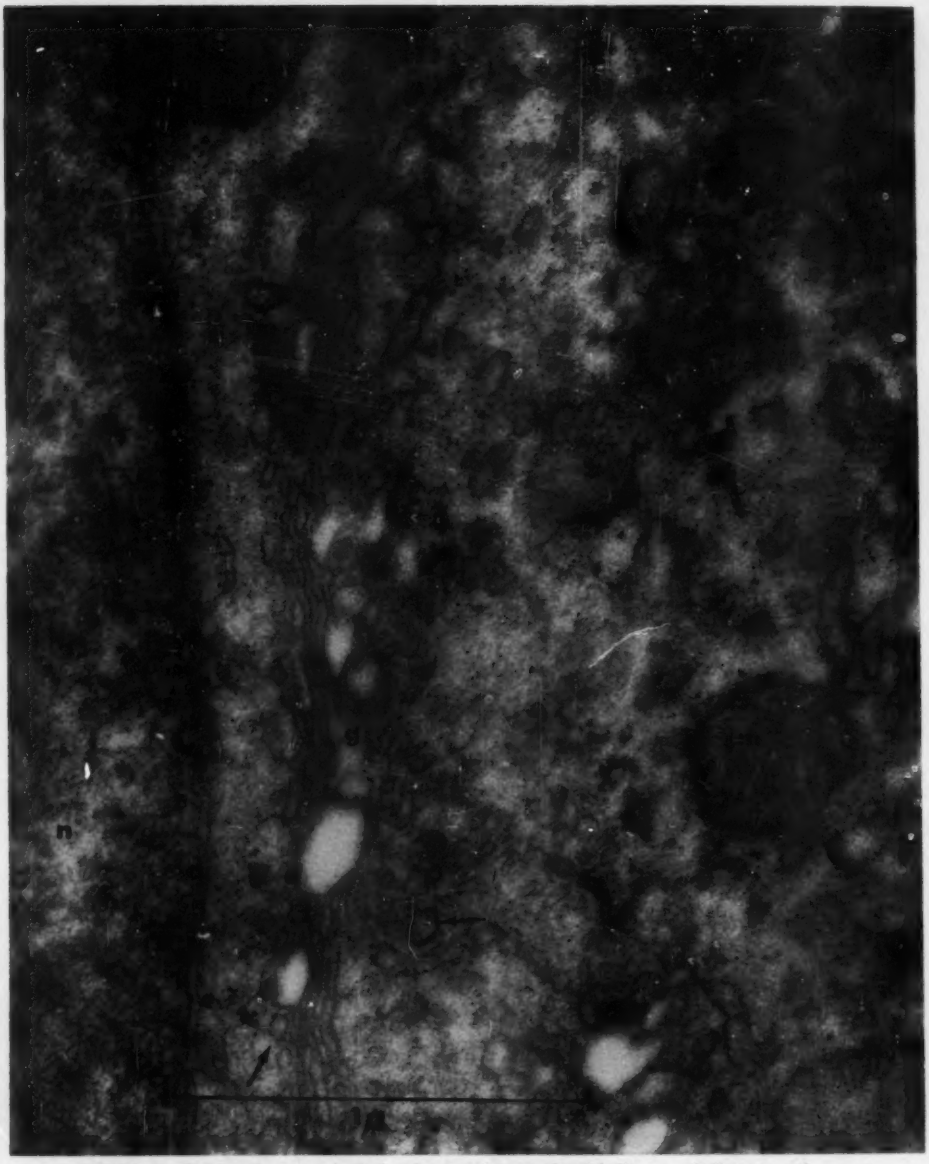
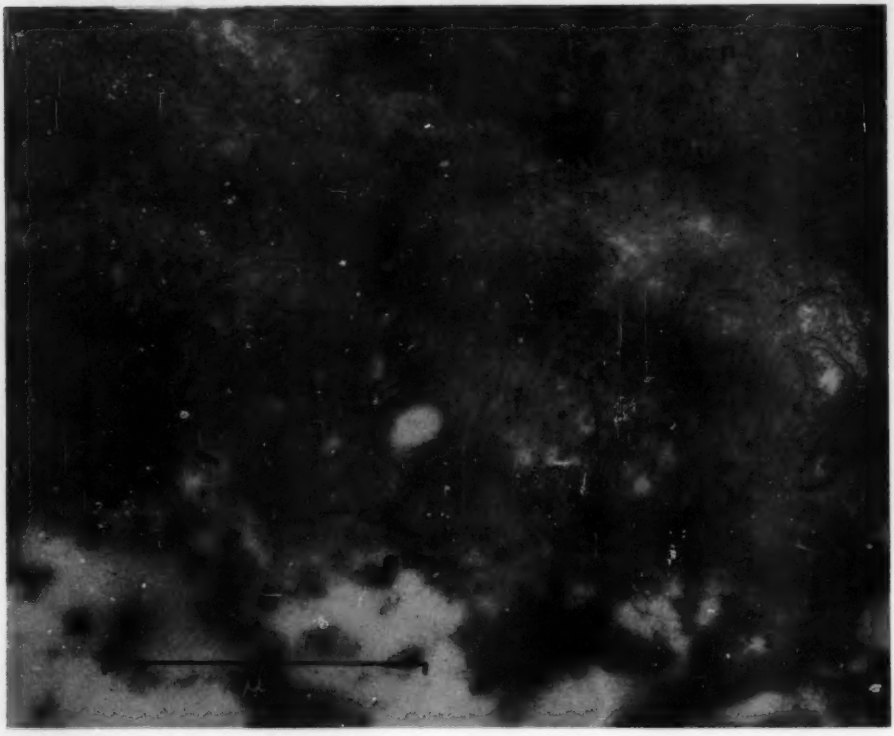


Fig. 11.—Golgi apparatus (*ga*) in the nonpigmented epithelium. *n* indicates nucleus; *m*, mitochondria. The arrows indicate small vesicles at the periphery of the membranes of the Golgi apparatus. Rabbit eye; × 67,000. Reproduced from Holmberg, A.: Thesis, Stockholm, 1957.

Fig. 13.—β-cytomembranes (β-*cm*) and vesicles arranged in rows (arrows) in the non-pigmented epithelium. *pc* indicates posterior chamber. Rabbit eye; × 94,000. Reproduced from Holmberg, A.: Thesis, Stockholm, 1957.

Fig. 12.—Mitochondria in the nonpigmented epithelium. *g* indicates granula. Rabbit eye; × 55,400.

Fig. 14.—β-cytomembranes (*β-cm*) in the pigment epithelium. *c* indicates ciliary stroma; *n*, nucleus. Note the thin external limiting membrane (*elm*) as compared with Figure 19. Rabbit eye; × 50,400.



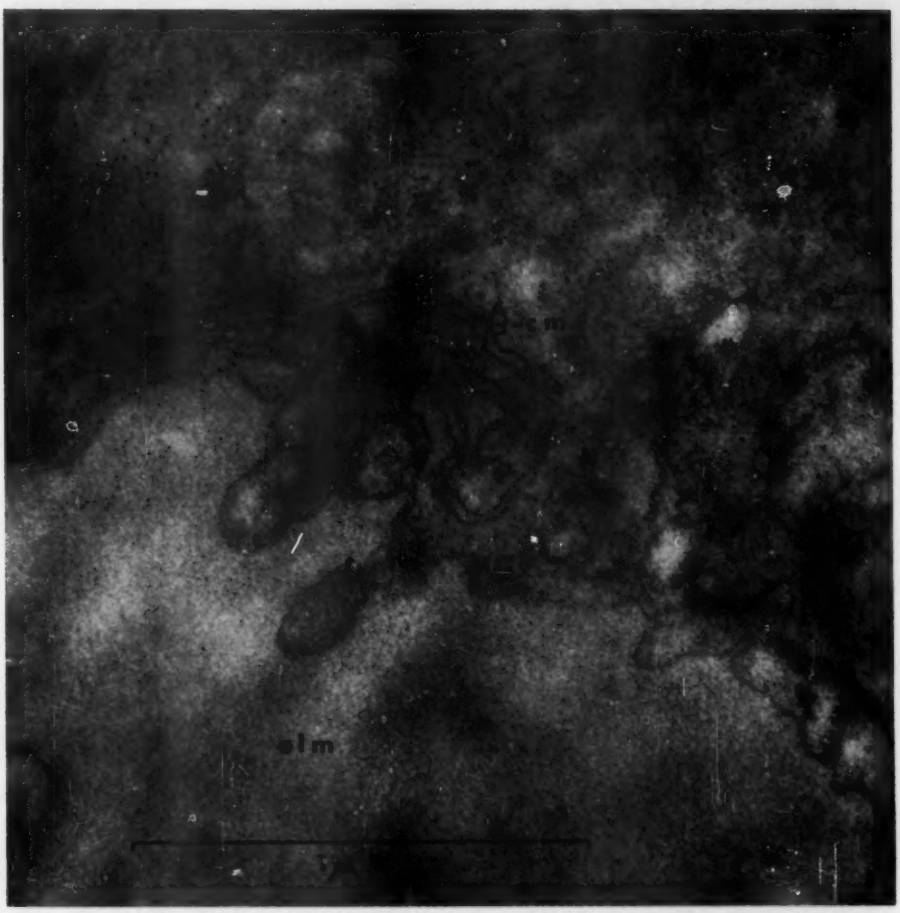


Fig. 15.—β-cytomembranes (β-*cm*) in the pigment epithelium. *elm* indicates external limiting membrane. Human eye; ×71,000.

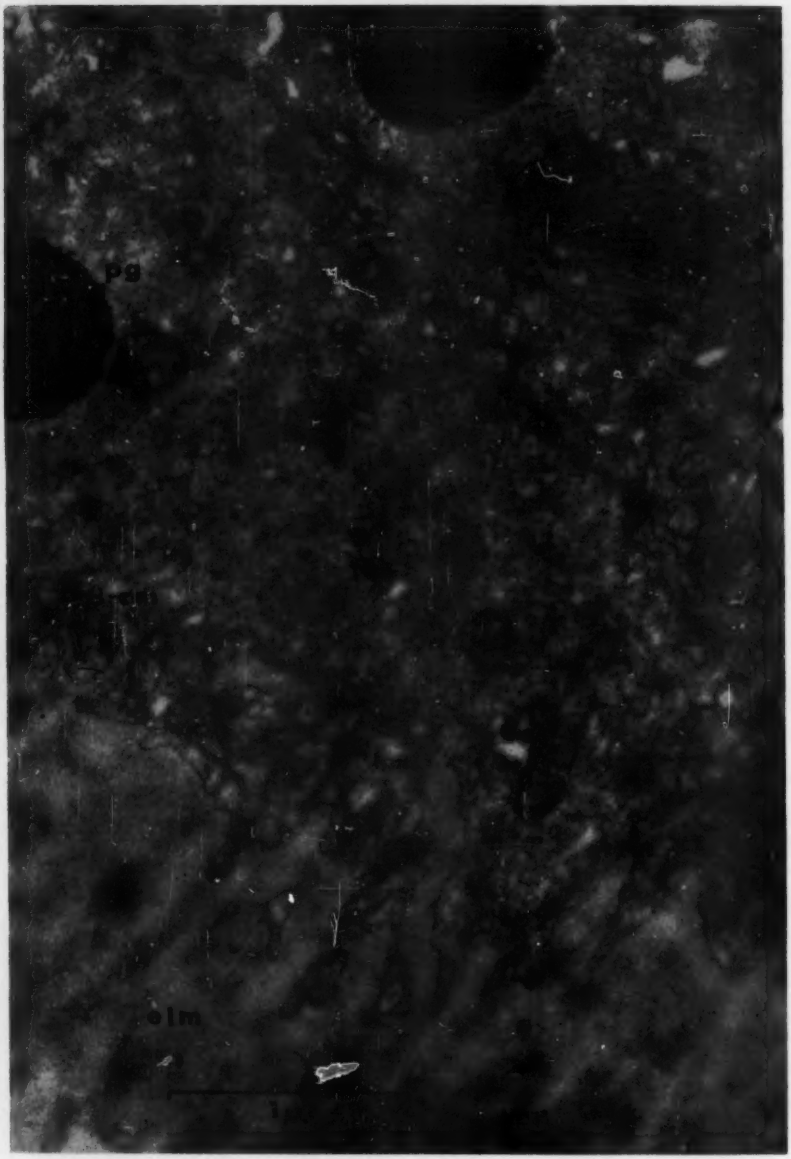


Fig. 16.—Basal part of the pigment epithelium with a very folded cell border (*cb*). *elm* indicates external limiting membrane; *β-cm*, β-cytomembranes; *pg*, pigment granules. Human eye; × 36,100.

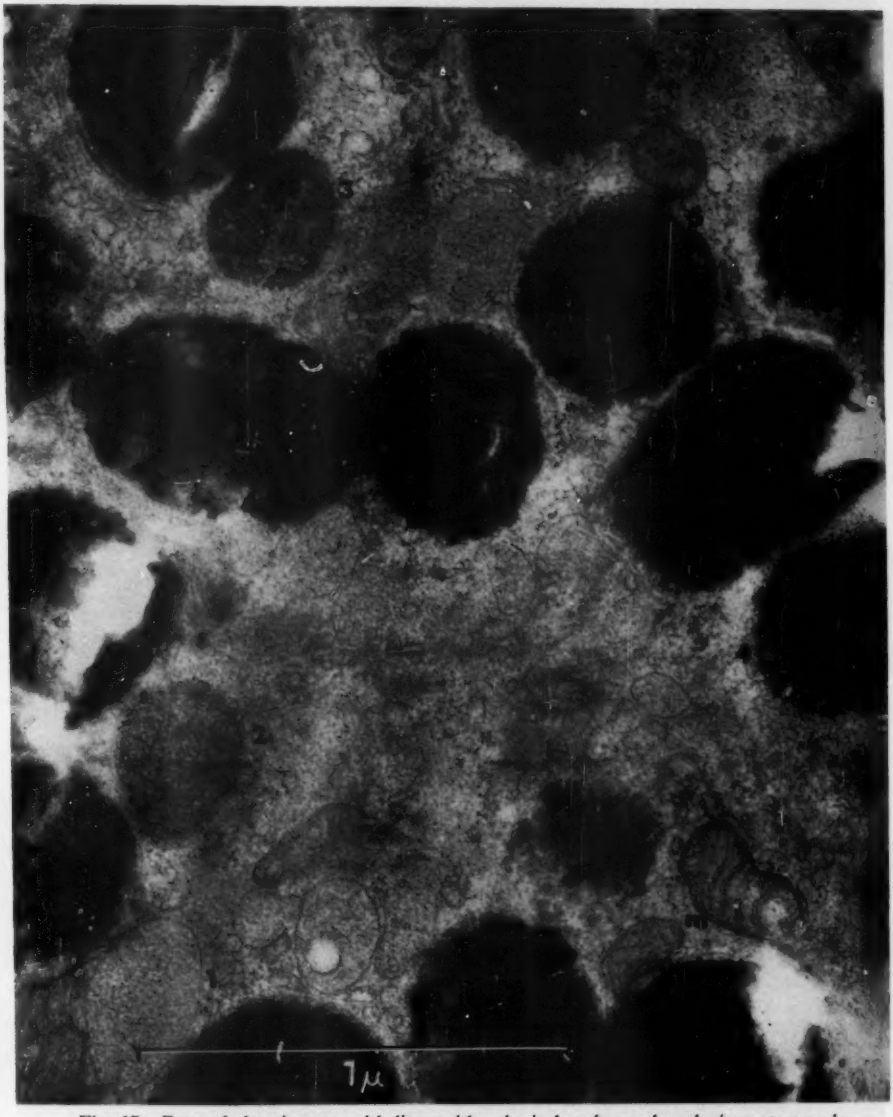


Fig. 17.—Part of the pigment epithelium with spherical and egg-shaped pigment granules. Note the difference in pigment content in the granules labelled 1-5. *m* indicates mitochondria. Rabbit eye; × 67,000.

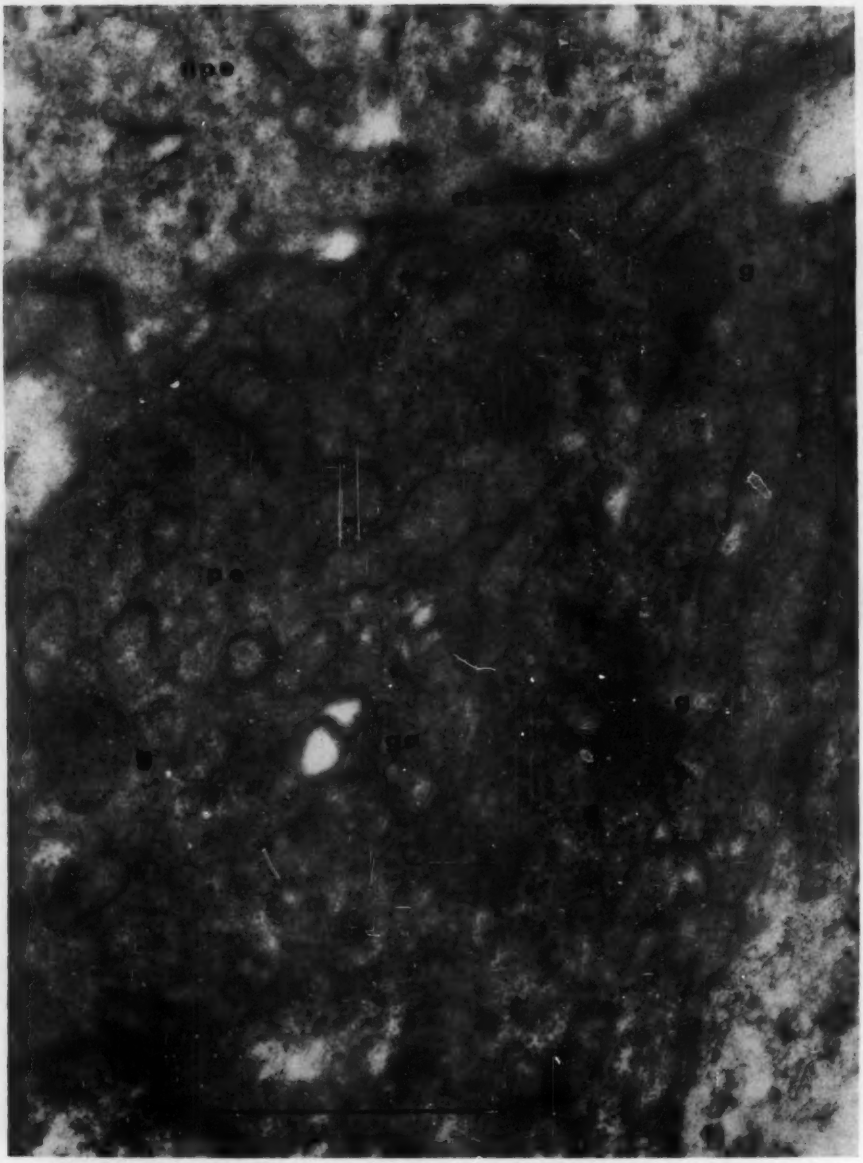


Fig. 18.—Pigment epithelium (*pe*) from an albino rabbit. There are several granules (*g*) with small black grains in their ground substance. *ga* indicates Golgi apparatus; *npe*, nonpigmented epithelium; *cb*, cell border. × 54,600.

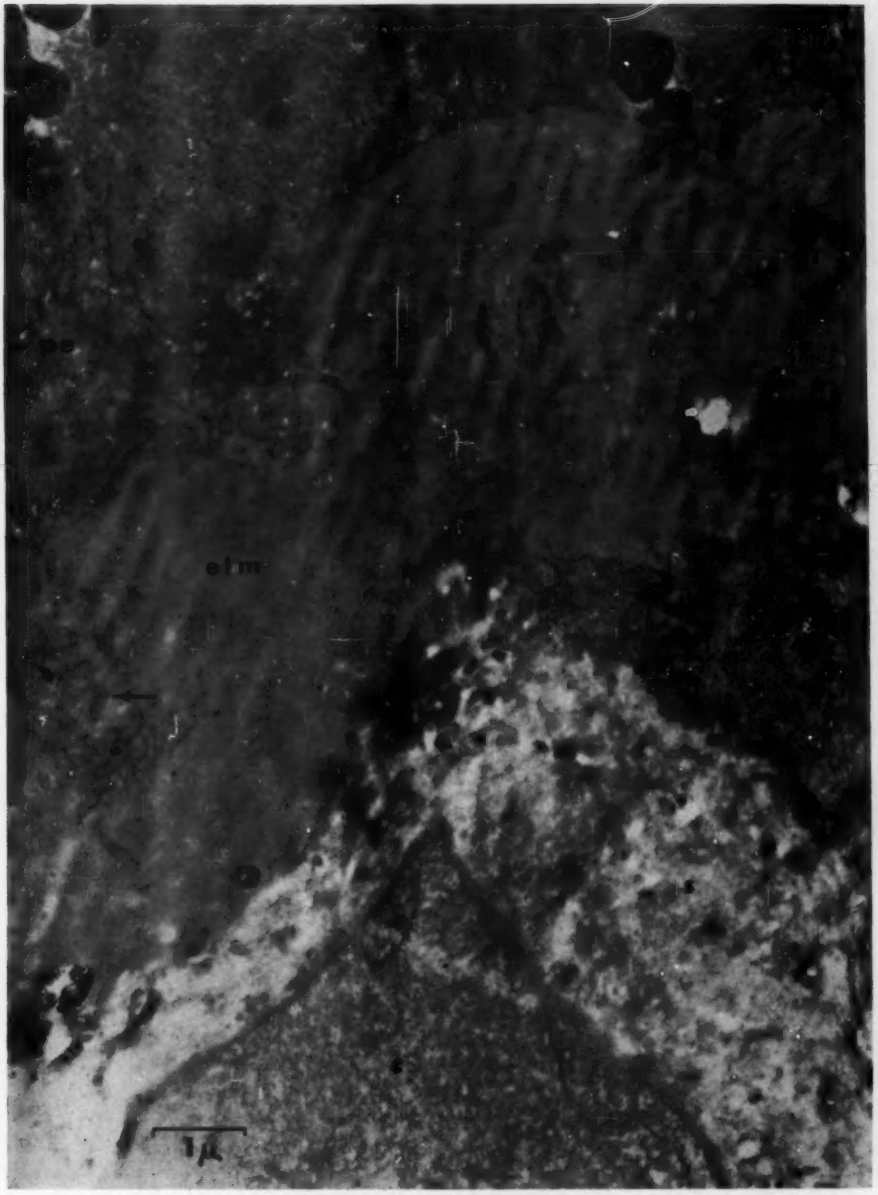


Fig. 19.—Survey picture of the central part of a ciliary process. *c* indicates capillary; *s*, ciliary stroma; *elm*, external limiting membrane; *pe*, basal part of the pigment epithelium with a nucleus (*n*). The surface of the epithelium is irregular with many cytoplasmic extensions (arrow). Human eye; × 14,500.

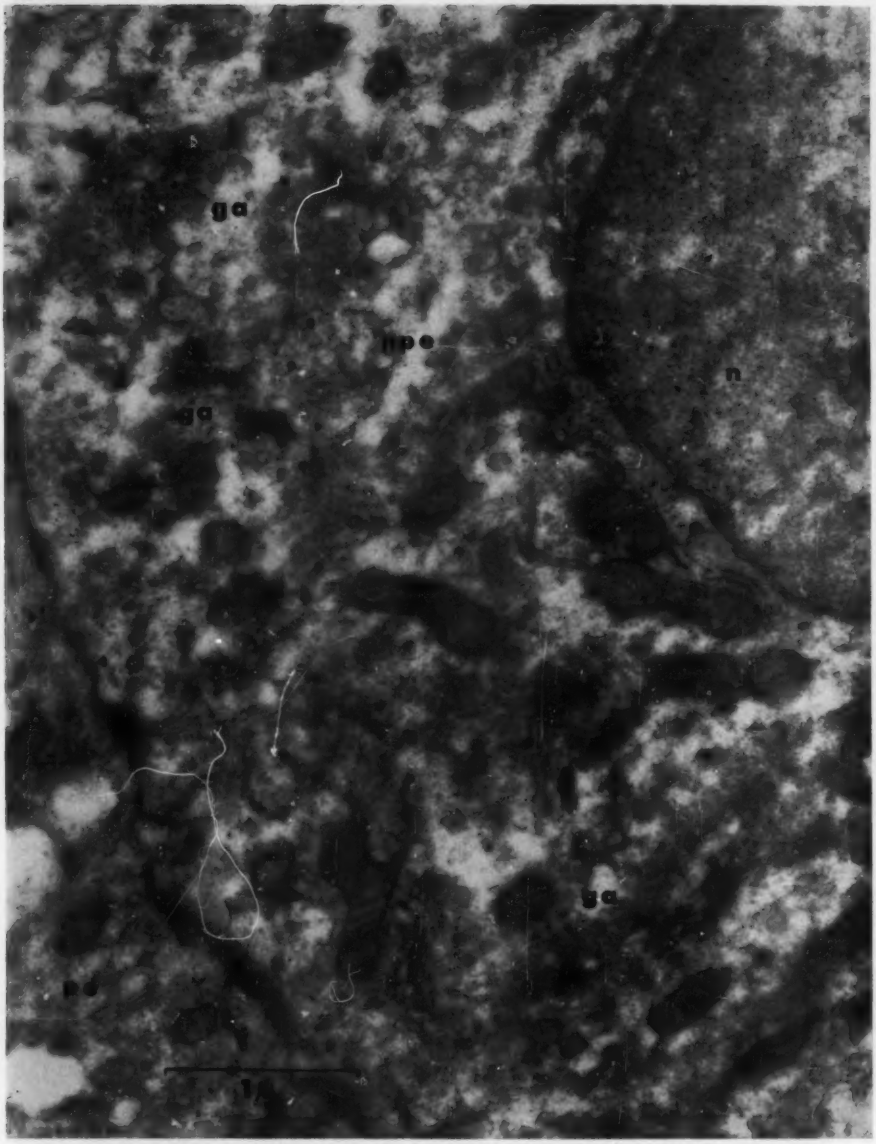


Fig. 20.—Basal part of the nonpigmented (*npe*) epithelium in a 30-day-old rabbit. Note the great number of vesicles in the Golgi region (*ga*). *n* indicates nucleus; *pe*, pigment epithelium. × 30,000.

Fig. 21.—The pigment epithelium (*pe*) and the nonpigmented epithelium (*npe*) in a one-day-old rabbit. *n* and n_2 indicate nuclei; *pc*, posterior chamber. Note the apical position of the nuclei in the nonpigmented epithelium. Note further the dense cytoplasm of the nonpigmented epithelium. × 8,000.

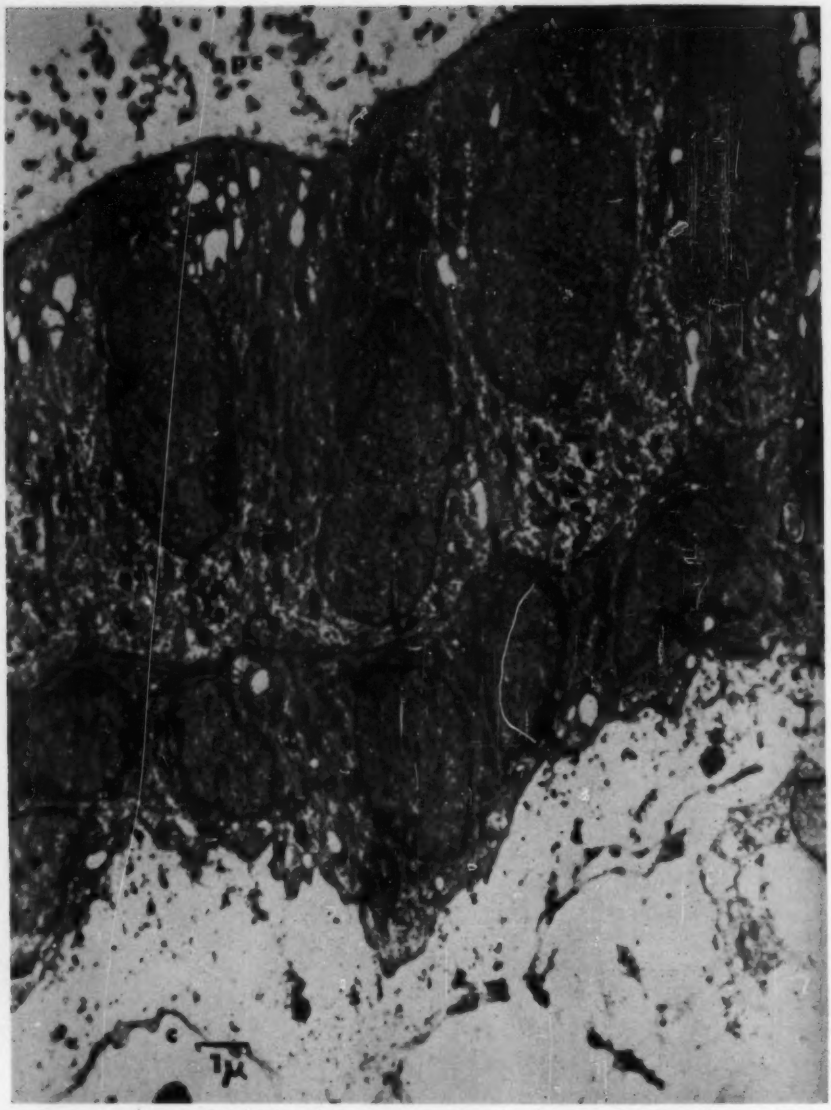


Fig. 22.—The pigment (*pe*) and the nonpigmented epithelium (*npe*) in a 30-day-old rabbit. *c* indicates capillary; *s*, ciliary stroma; *n* and n_1, nuclei; *pc*, posterior chamber. The nuclei in the nonpigmented epithelium have their usual basal location, and the cytoplasm of the nonpigmented epithelium is less dense. × 8,000.

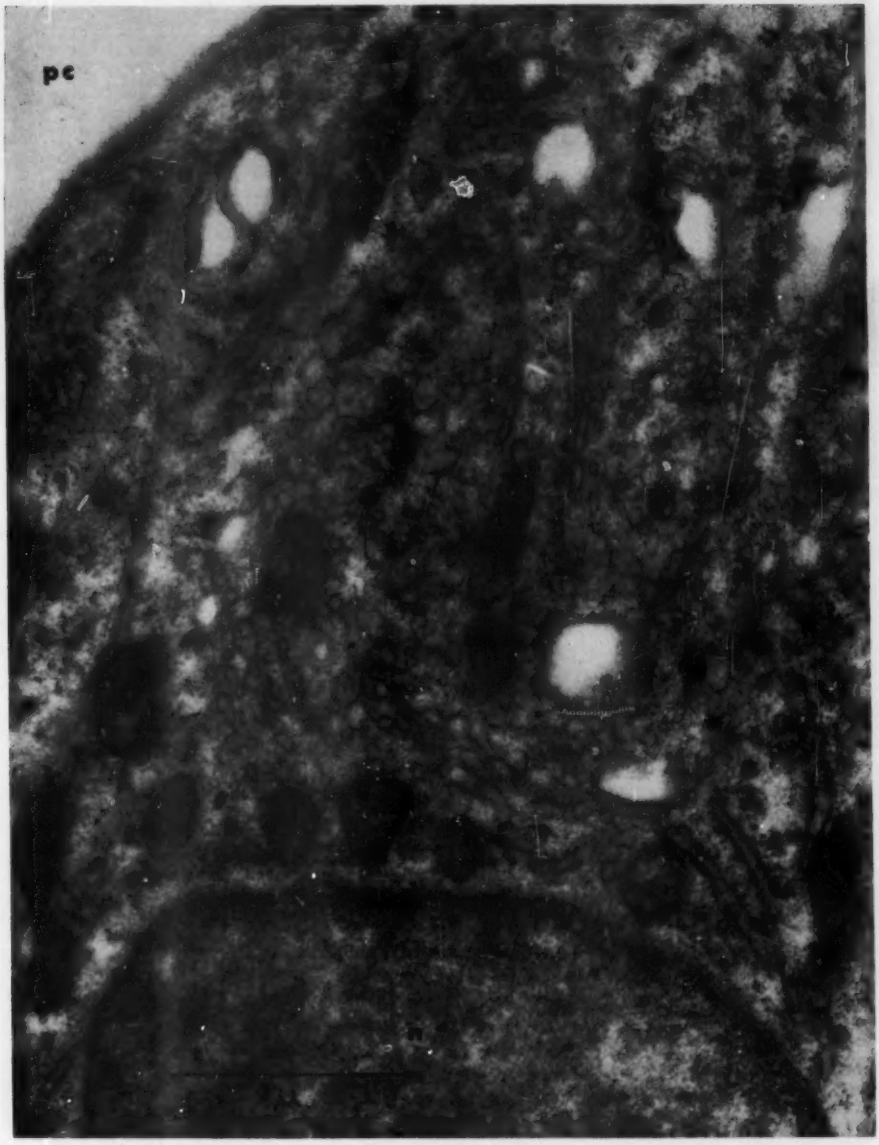


Fig. 23.—Apical part of the nonpigmented epithelium in a 30-day-old rabbit. There are a large number of vesicles arranged in rows in the cytoplasm above the nucleus (*n*). *pc* indicates posterior chamber. × 39,000.

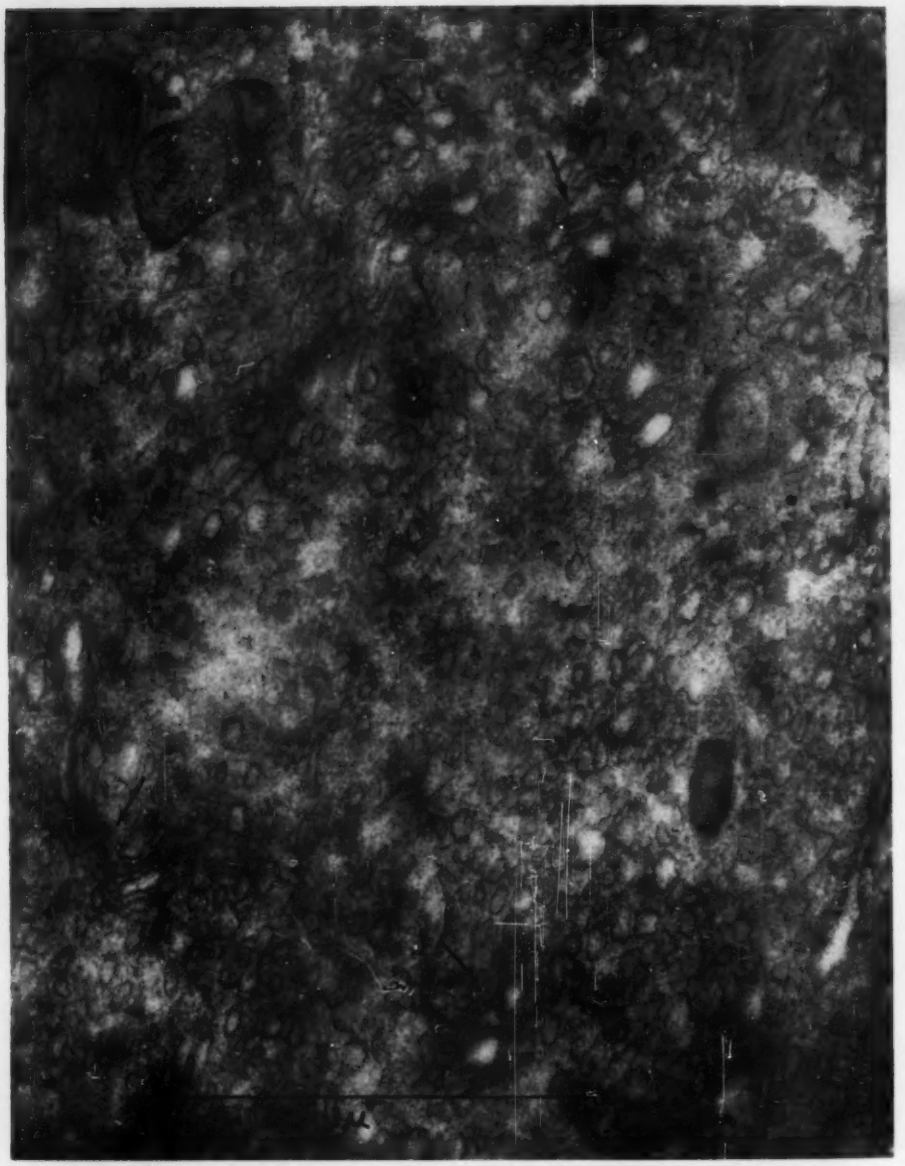


Fig. 24.—Golgi apparatus (arrows) 15 minutes after intravenous administration of acetazolamide (10 mg. per kilogram). Note the large number of vesicles and the short membranes in the Golgi region. *m* indicates mitochondria. Compare Figure 11. Rabbit eye; × 72,000.

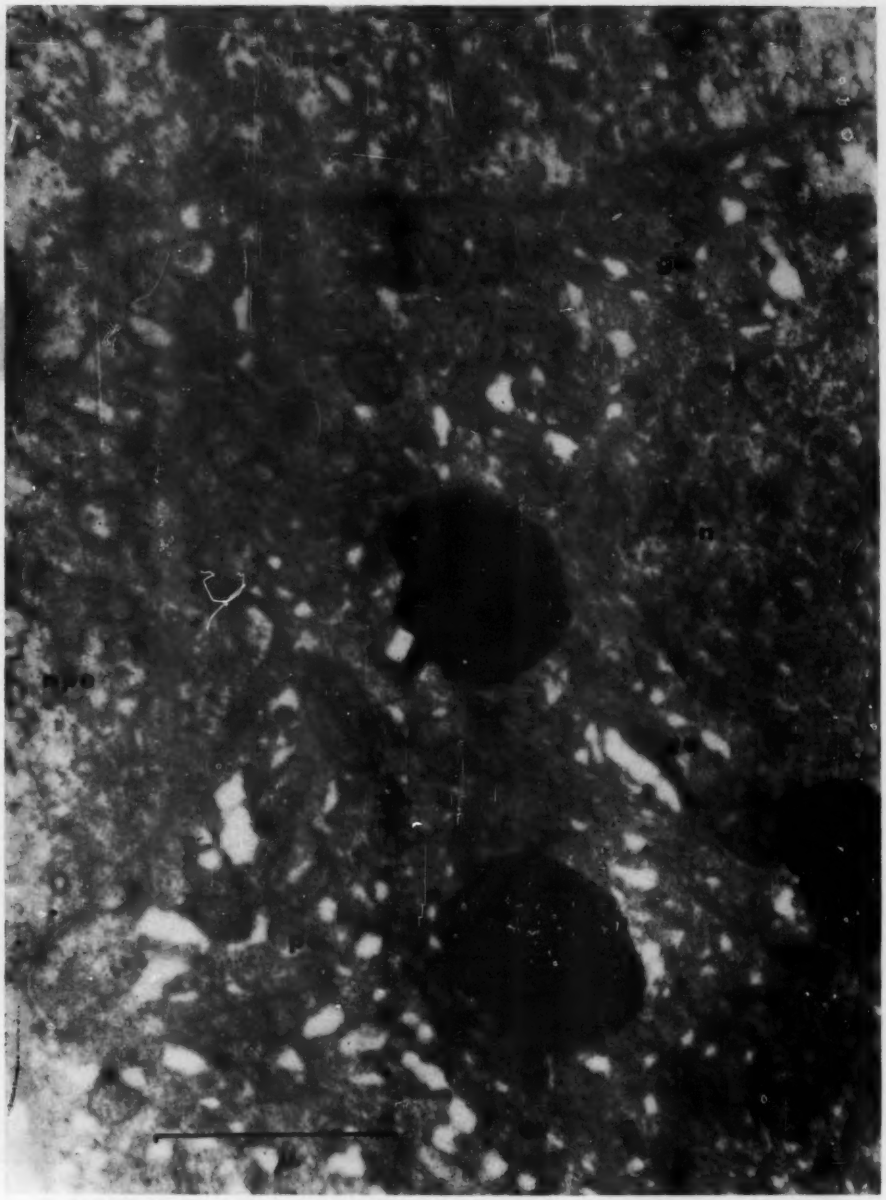


Fig. 25.—Part of the pigment (*pe*) and nonpigmented epithelium (*npe*) 15 minutes after intravenous administration of acetazolamide (500 mg.). Note the large number of vesicles in the Golgi region (*ga*). Human eye; × 37,000.

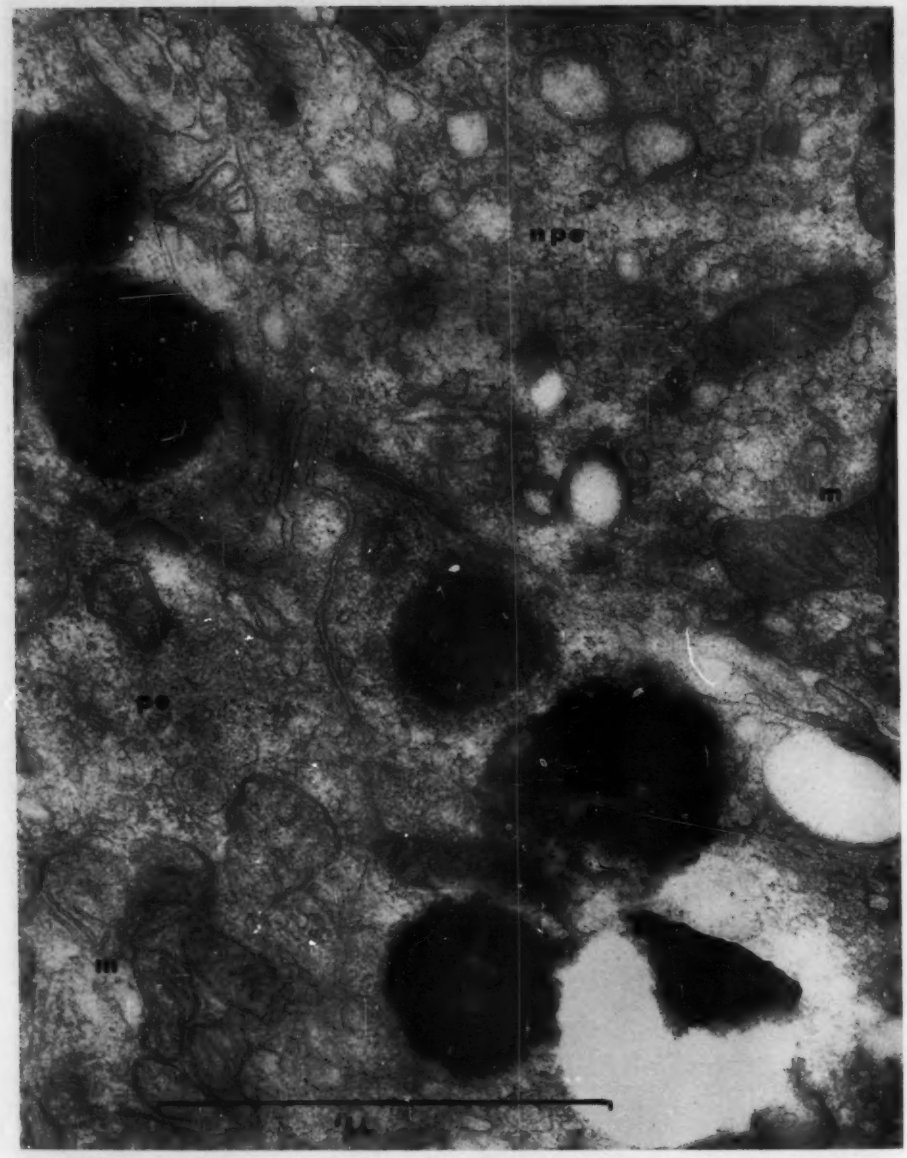


Fig. 26.—Part of the pigment (*pe*) and nonpigmented epithelium (*npe*) 15 minutes after intravenous administration of acetazolamide (10 mg. per kilogram). Note the difference in the number of vesicles in the two epithelia. *m* indicates mitochondria; *cb*, cell border. Rabbit eye, × 71,000.

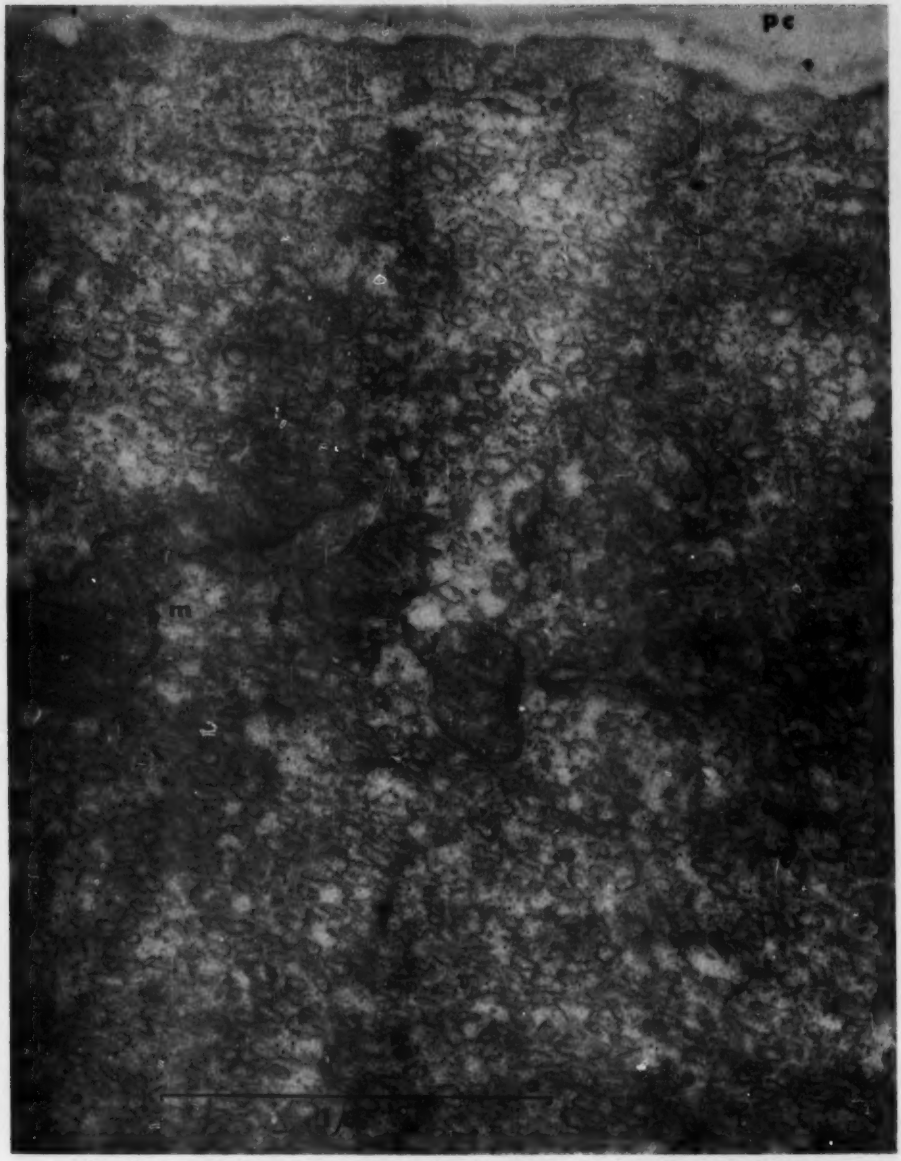


Fig. 27.—Part of the nonpigmented epithelium 15 minutes after intravenous administration of acetazolamide (10 mg. per kilogram) showing the enormous accumulation of vesicles in the cytoplasm. *m* indicates mitochondria; *pc*, posterior chamber. Rabbit eye; × 61,000. Reproduced from Holmberg, A.: Thesis, Stockholm, 1957.

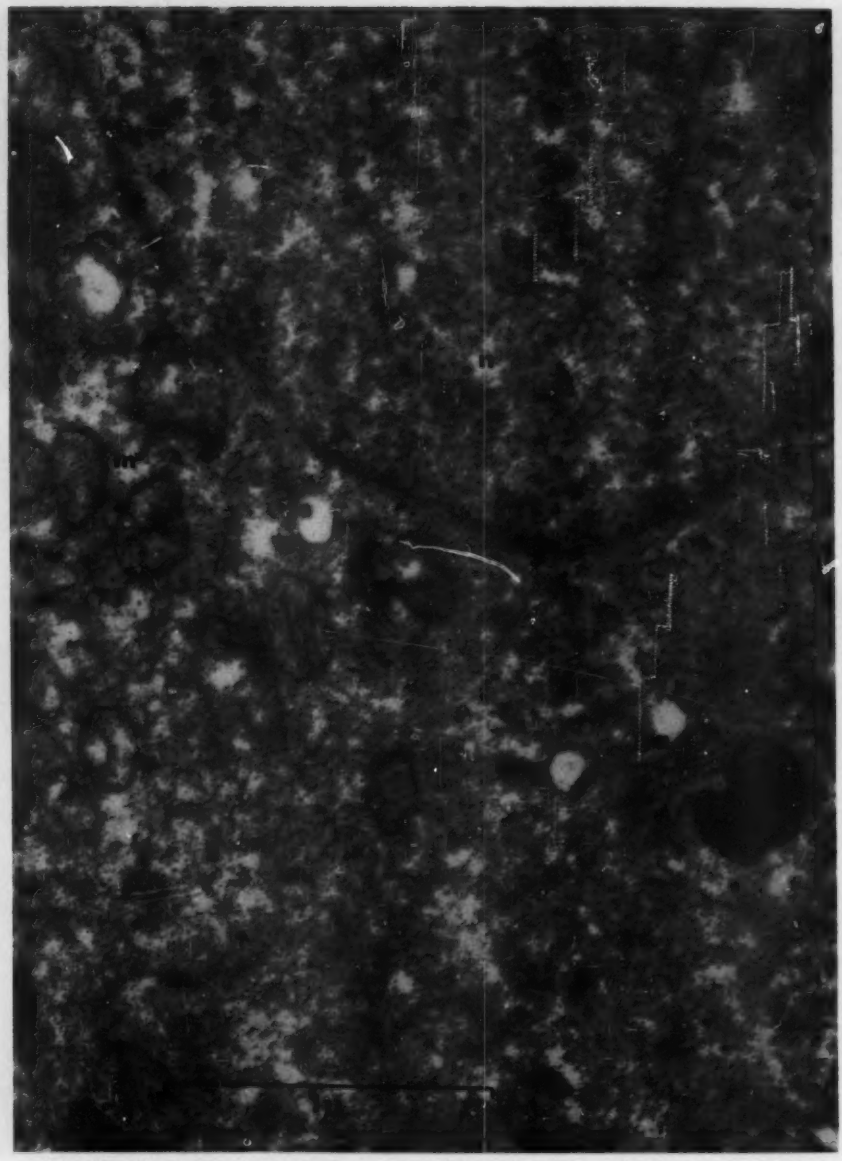


Fig. 28.—Basal part of the nonpigmented epithelium 15 minutes after intravenous injection of acetazolamide (500 mg.). As compared with Figure 27, there is no increase in the number of vesicles in the cytoplasm. *ga* indicates Golgi apparatus; *m*, mitochondria; *n*, nucleus. Human eye; × 51,600.

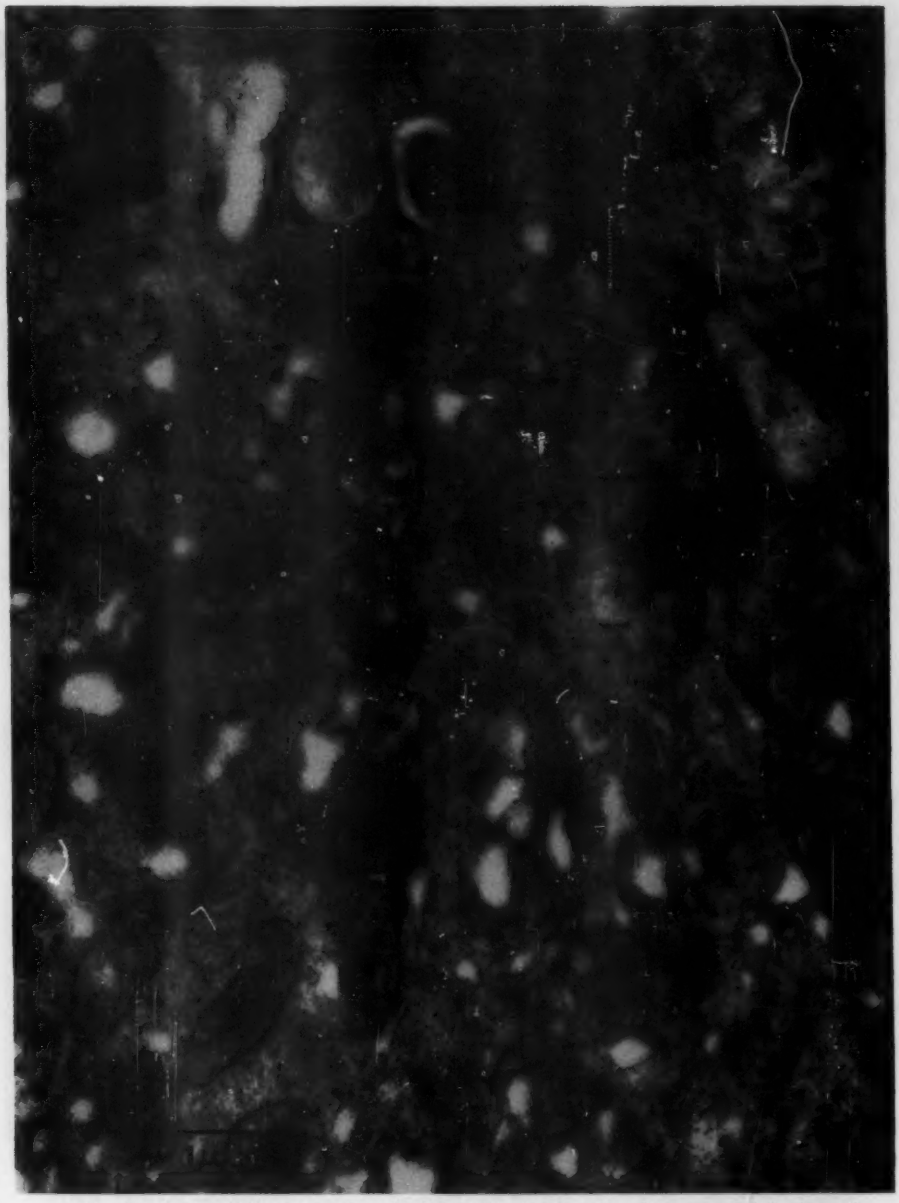


Fig. 29.—Part of the pigment epithelium 15 minutes after intravenous injection of acetazolamide (500 mg.). Note the large number of vesicles in the cytoplasm. Human eye; × 43,200.

Fig. 30.—Part of the nonpigmented epithelium 30 minutes after intravenous administration of acetazolamide (10 mg. per kilogram). The vesicles are regularly arranged in rows (arrows). *n* indicates nucleus; *m*, mitochondria; *β-cm*, β-cytomembranes; *pc*, posterior chamber. Rabbit eye; × 56,000. Reproduced from Holmberg, A.: Thesis, Stockholm, 1957.

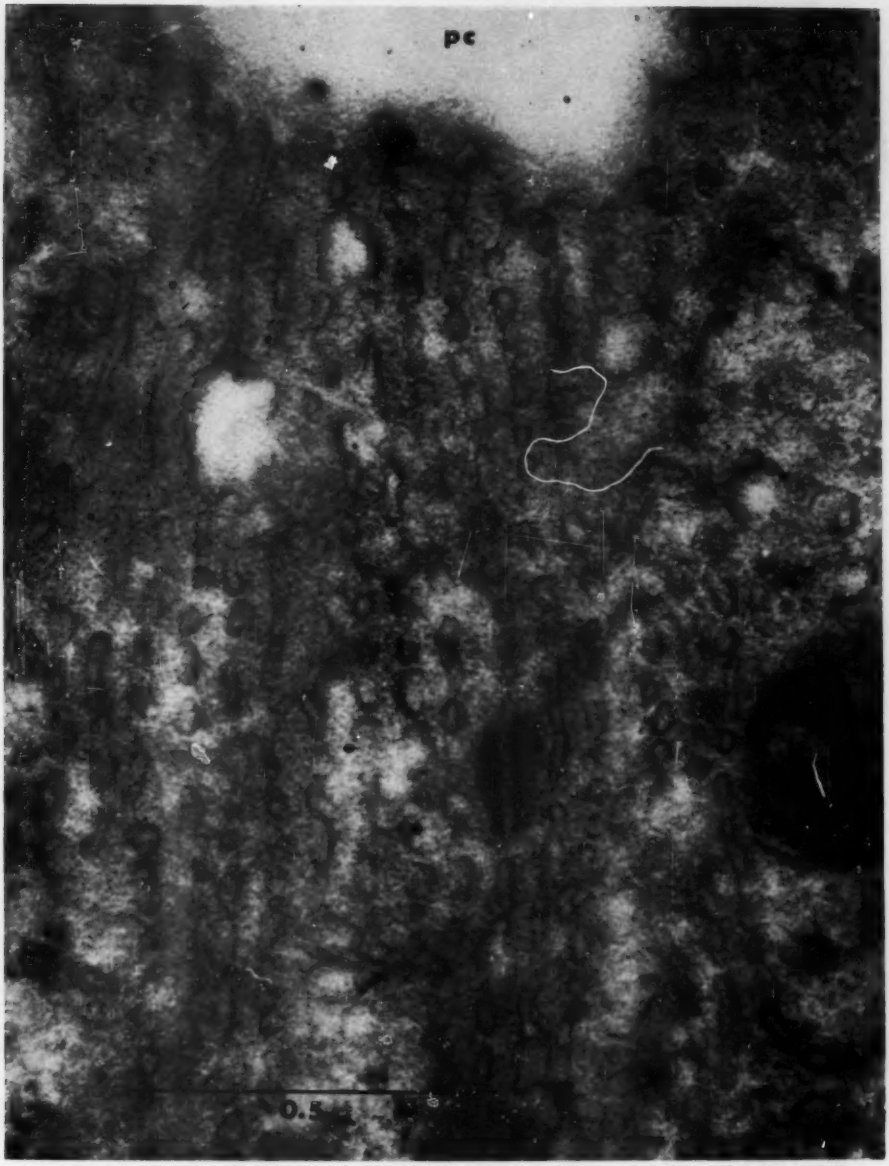


Fig. 31.—Part of the nonpigmented epithelium 60 minutes after intravenous administration of acetazolamide (10 mg. per kilogram) showing the regular arrangement of the vesicles. *pc* indicates posterior chamber. Rabbit eye; × 110,000. Reproduced from Holmberg, A.: Thesis, Stockholm, 1957.

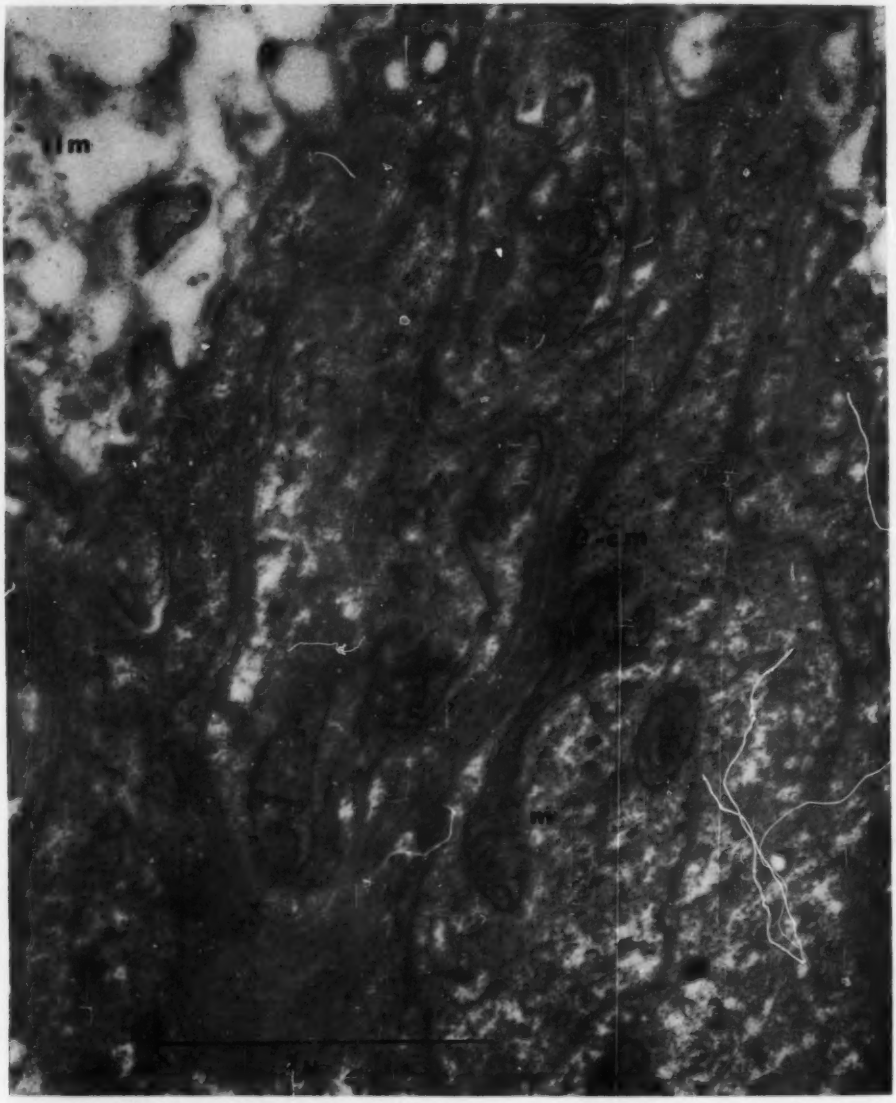


Fig. 32.—Part of the nonpigmented epithelium 15 minutes after intravenous administration of acetazolamide (500 mg.). There is no change in the normal structure. Compare Figures 30 and 31. *m* indicates mitochondria; *β-cm*, β-cytomembranes; *ilm*, internal limiting membrane. Human eye; × 52,000.

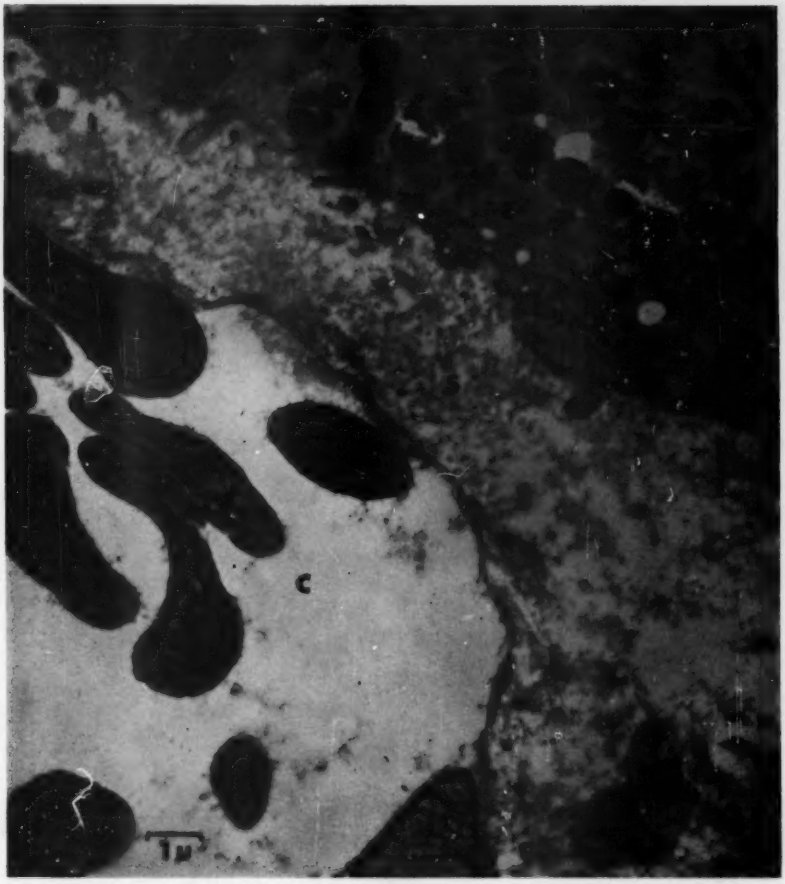


Fig. 1.—Survey picture of the central portion of a ciliary process with a part of a capillary (*C*), ciliary stroma (*S*), and the pigment epithelium (*PE*). Rabbit eye; × 10,000.

The figures on pp. 1033 to 1036 are for the article on pp. 949 to 951 by Holmberg.

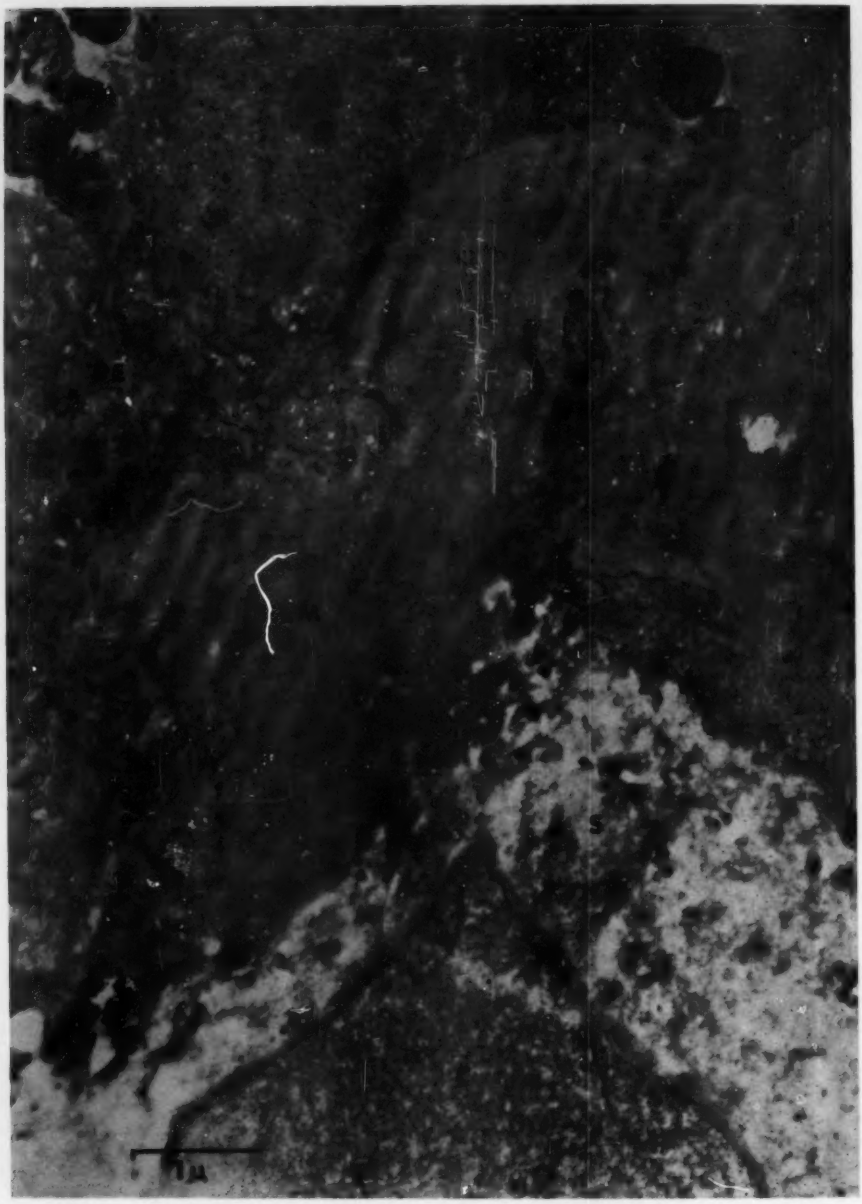


Fig. 2.—Survey picture from a human eye showing the relationship between the capillary (*C*), the external limiting membrane (*ELM*), and the pigment epithelium (*PE*); × 20,000.

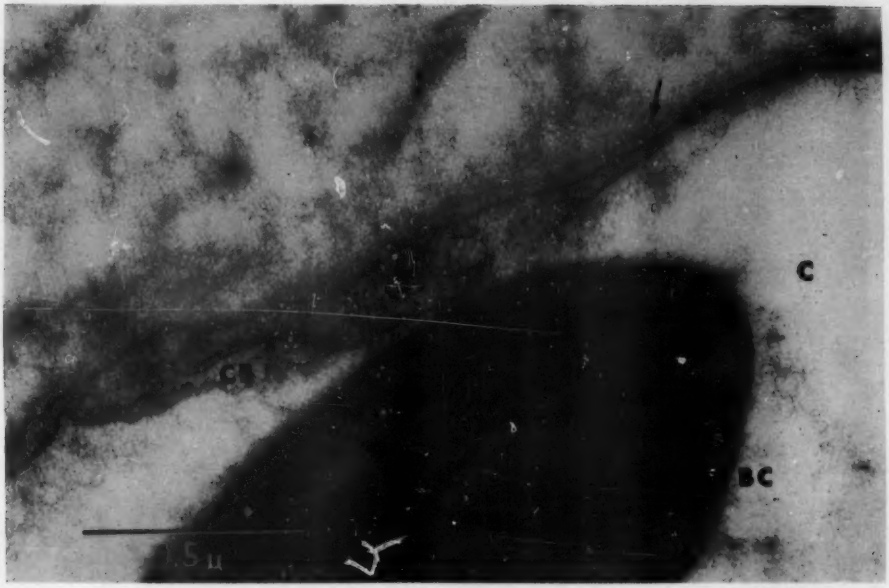
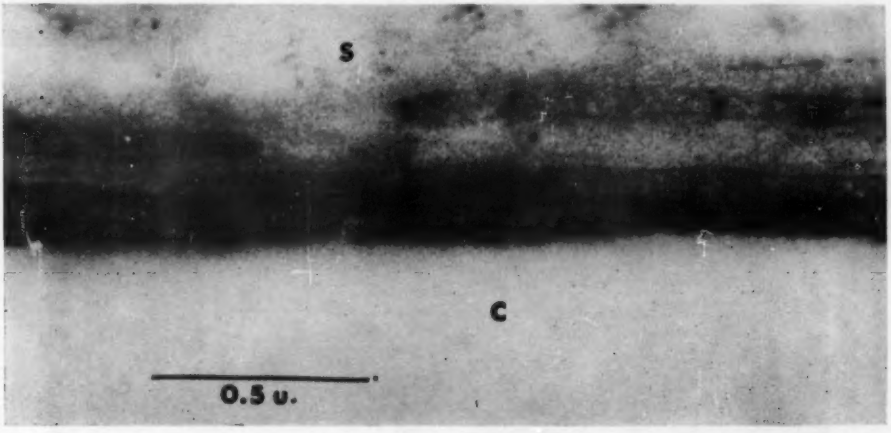


Fig. 3.—Extremely thin capillary wall with a basement membrane (arrow). Where two cells border each other there is an overlapping (*CB*). C=capillary lumen. BC=red blood corpuscle. S=ciliary stroma. Rabbit eye; × 70,000.

Fig. 4.—Capillary wall with two endothelial cells overlapping each other. C=capillary lumen. S=ciliary stroma. Rabbit eye; × 67,000.



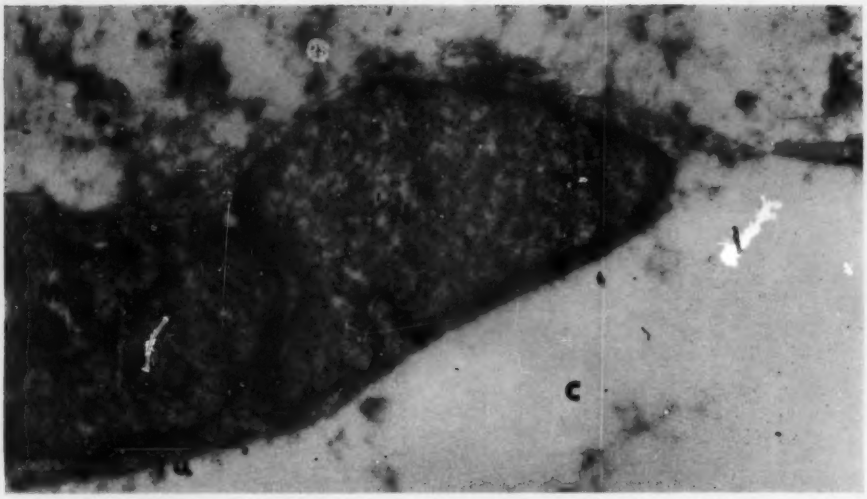


Fig. 5.—Capillary endothelium with an elongated nucleus (*N*). Some mitochondria (*M*) and a Golgi apparatus (*GA*) are visible in the cytoplasm. C=capillary lumen. S=ciliary stroma. Rabbit eye; × 20,000.

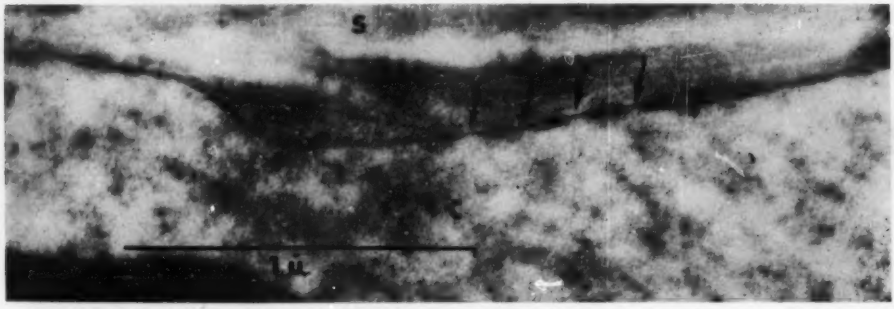


Fig. 6.—Capillary wall with several pores (*arrows*). C=capillary lumen. S=ciliary stroma. Rabbit eye; magnification × 55,000.

Fig. 7.—Capillary wall with two pores (*arrows*). C=capillary lumen. BC=part of a red blood corpuscle. S=ciliary stroma. Rabbit eye; × 84,000.

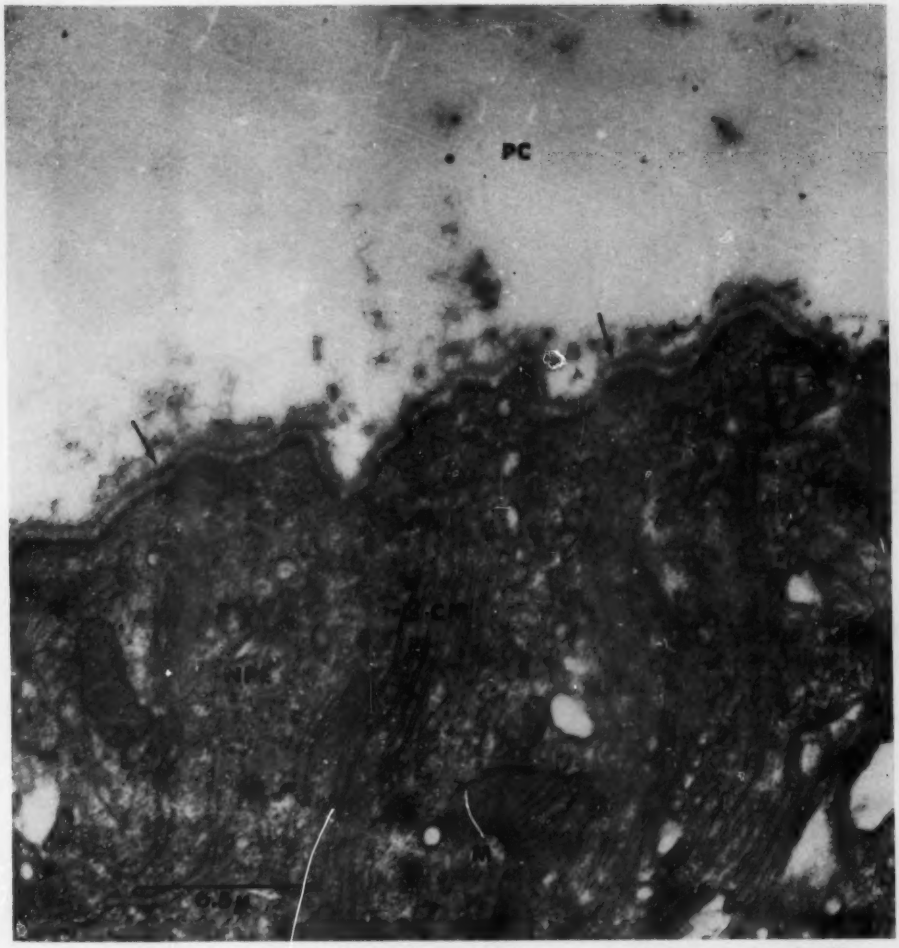


Fig. 1.—Apical part of a nonpigmented cell (*NPE*) from a rabbit eye showing the thin internal limiting membrane (arrows). In the cytoplasm mitochondria (*M*) and β-cytomembrane (*β-CM*) are visible. *PC* indicates the posterior chamber; × 56,000.

The figures on pp. 1037 to 1046 are for the article on pp. 952 to 955 by Holmberg.

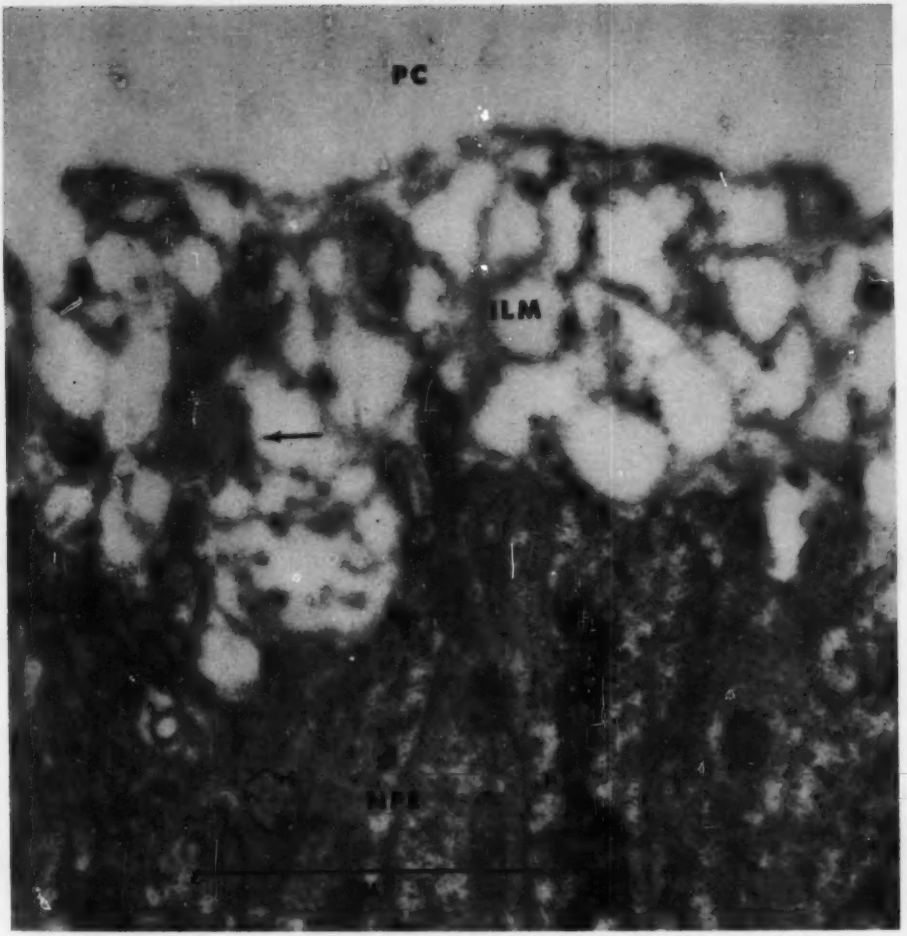


Fig. 2.—Apical part of a nonpigmented cell (*NPE*) from a human eye. Note the thick internal limiting membrane (*ILM*), which appears as a meshwork. Occasionally some of the meshes are filled with a homogenous material (*arrow*) *PC* indicates the posterior chamber; × 54,000.

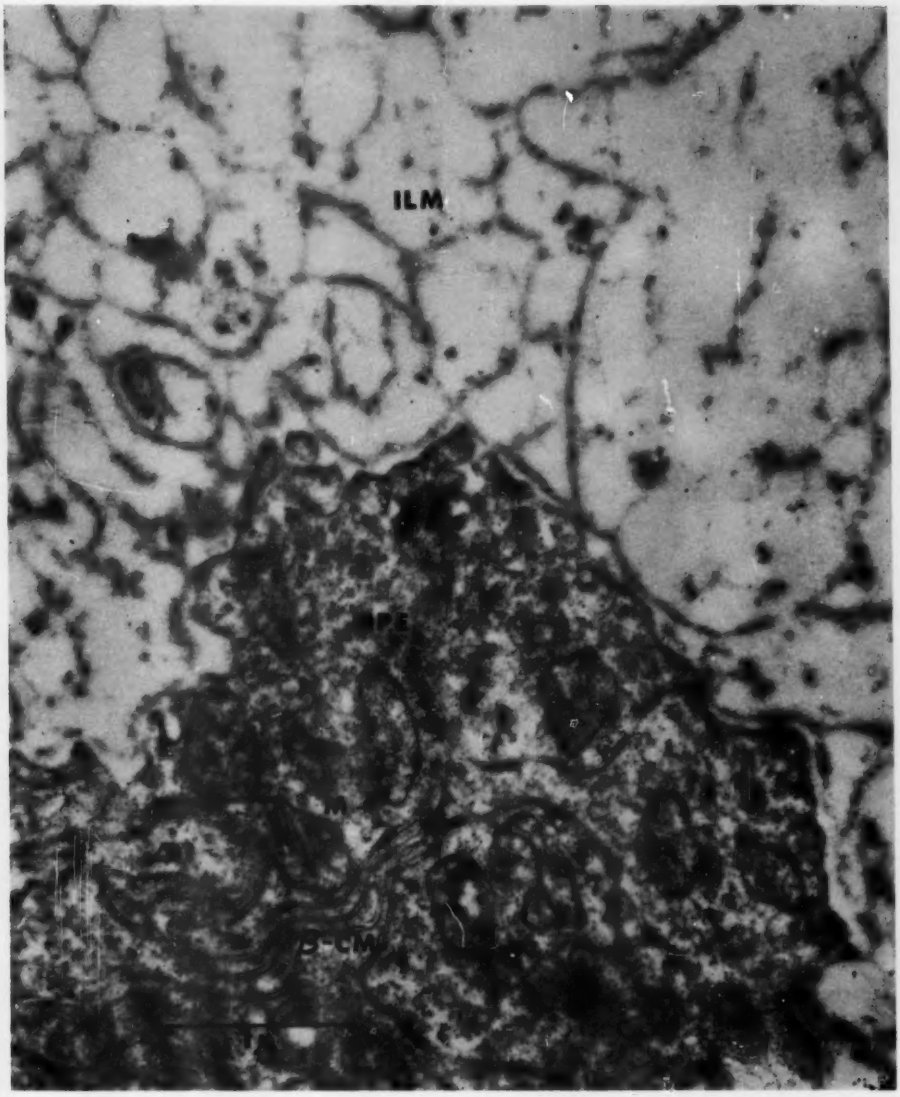


Fig. 3.—Apical part of a nonpigmented cell (*NPE*) from a human eye. The meshwork of the internal limiting membrane (*ILM*) is clearly visible. In the cytoplasm mitochondria (*M*) and β-cytomembranes (*β-CM*) are visible; × 30,000.

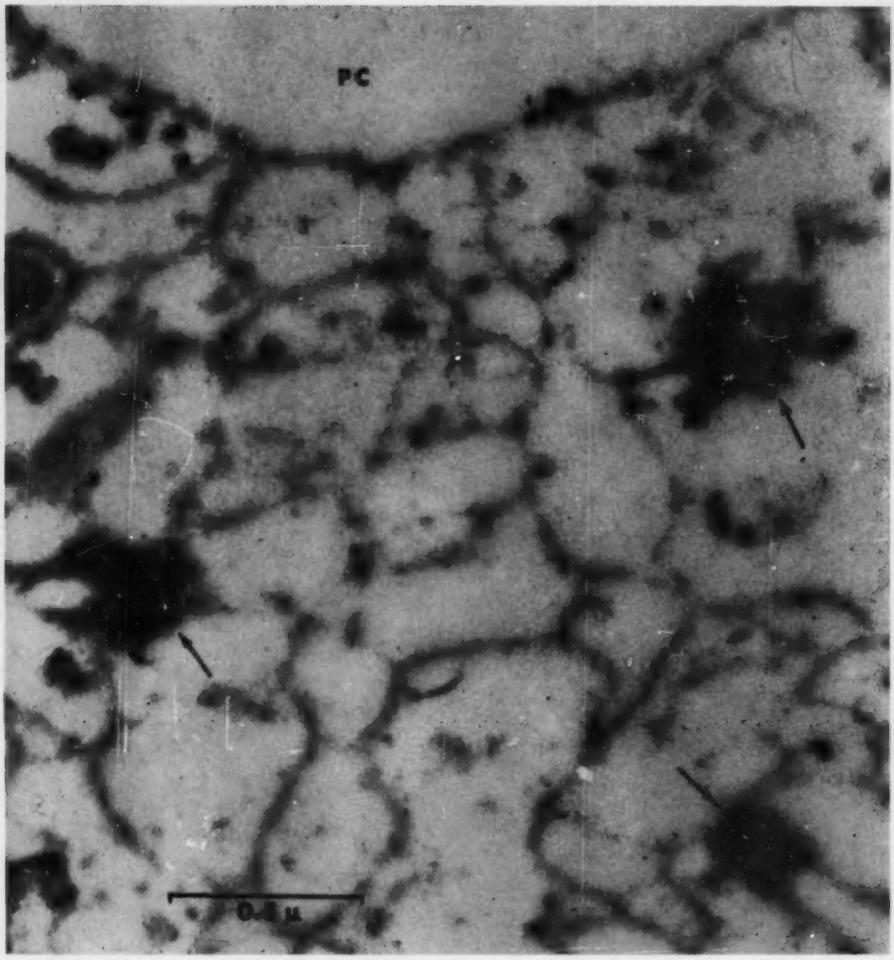


Fig. 4.—Higher magnification of the internal limiting membrane from a human eye. The membrane is well defined from the posterior chamber (*PC*) by a continuous membrane. In the meshes there are sometimes encountered a dense homogenous substance (*arrows*). *PC* indicates the posterior chamber; × 60,000.

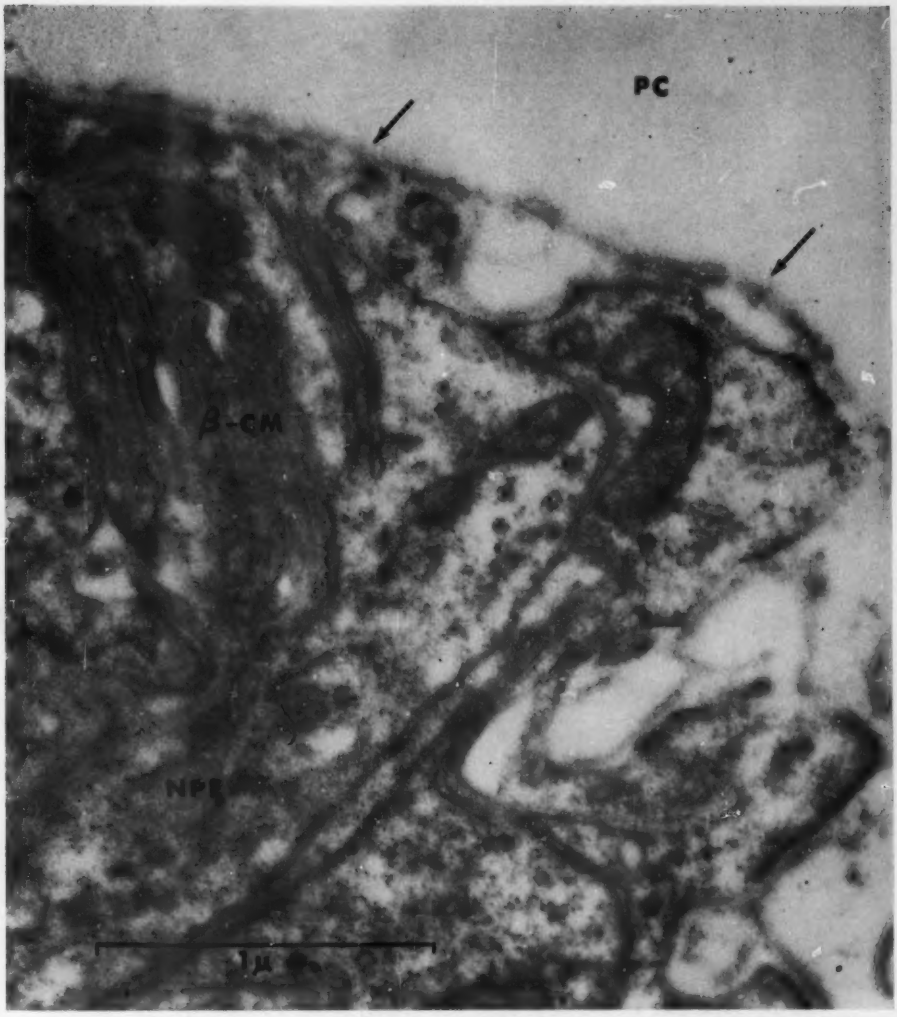


Fig. 5.—Apical part of a nonpigmented cell (*NPE*) from a human eye. Within restricted areas the internal limiting membrane is reduced in thickness to a single layer (*arrows*) as is the normal appearance of the same membrane in rabbit eye. Some β-cytomembranes (*β-CM*) appear in the cytoplasm; × 53,000.

Fig. 6.—Apical part of a nonpigmented cell (*NPE*) from a human eye. The internal limiting membrane (*ILM*) is always found in the indentation between two adjacent cells. *β-CM* indicates β-cytomembranes in the cytoplasm; × 46,000.

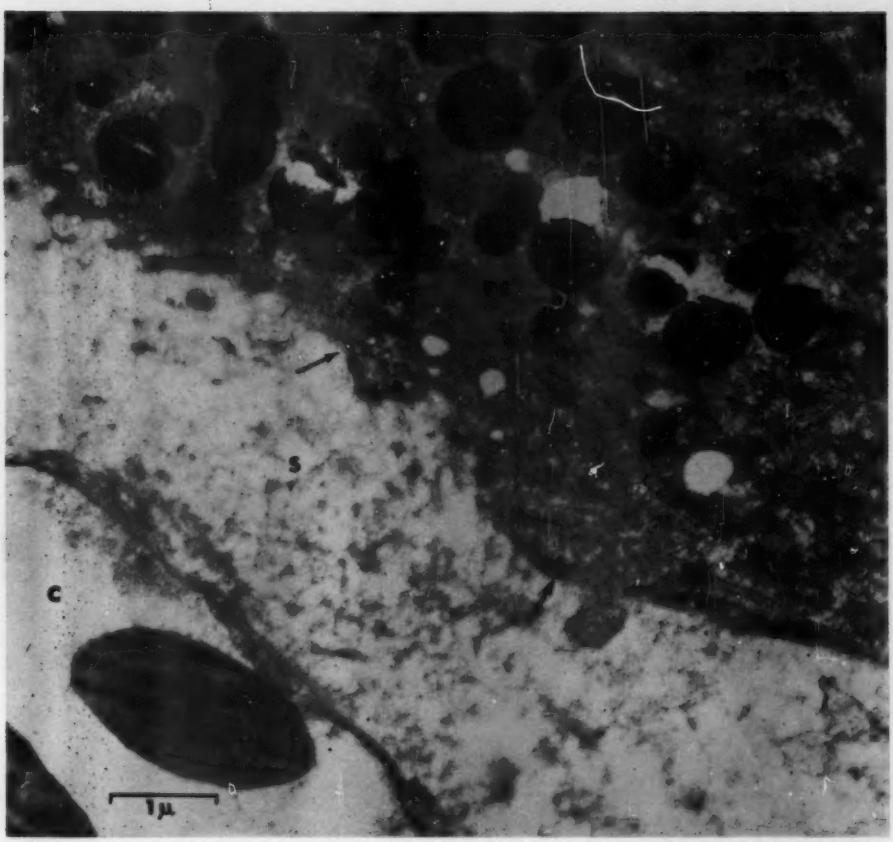


Fig. 7.—Survey picture of the basal part of a ciliary process in a rabbit eye. In the left lower corner a part of a capillary (*C*) is seen with red blood cells and a thin capillary wall. The ciliary stroma (*S*) occupies the space between the capillary and the pigmented epithelium (*PE*). The arrows point to the external limiting membrane. *NPE* indicates the nonpigmented epithelium; × 18,000.

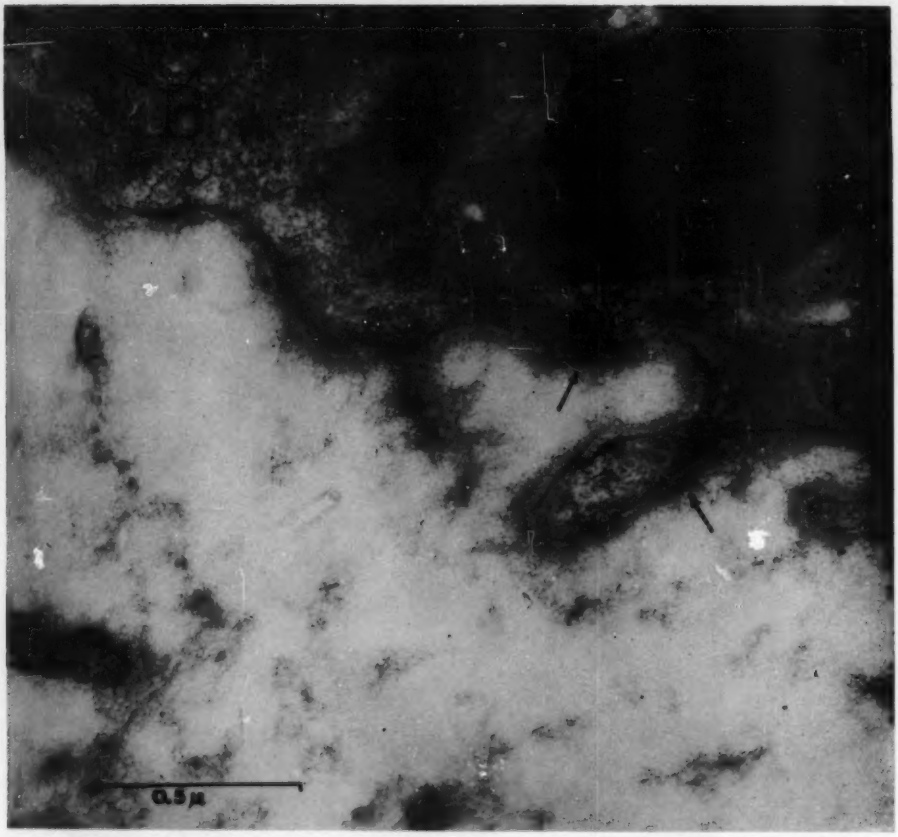


Fig. 8.—High magnification of the most basal part of a pigmented cell (*PE*) from a rabbit eye. The arrows indicate the position of the very thin external limiting membrane. In the cytoplasm mitochondria (*M*) and a pigment granule are visible; × 68,000.

Fig. 9.—Survey picture of the basal part of a ciliary process from a human eye. A capillary (*C*) and the stroma (*S*) occupy the lower part of the picture. Between the stroma and the pigmented epithelium (*PE*) there is a surprisingly thick external limiting membrane (*ELM*). The surface of the pigmented cells is very irregular with large cytoplasmic extensions. Two nuclei (*N*) and pigment granules (*PG*) are seen in the pigmented cells. Magnification × 20,000.

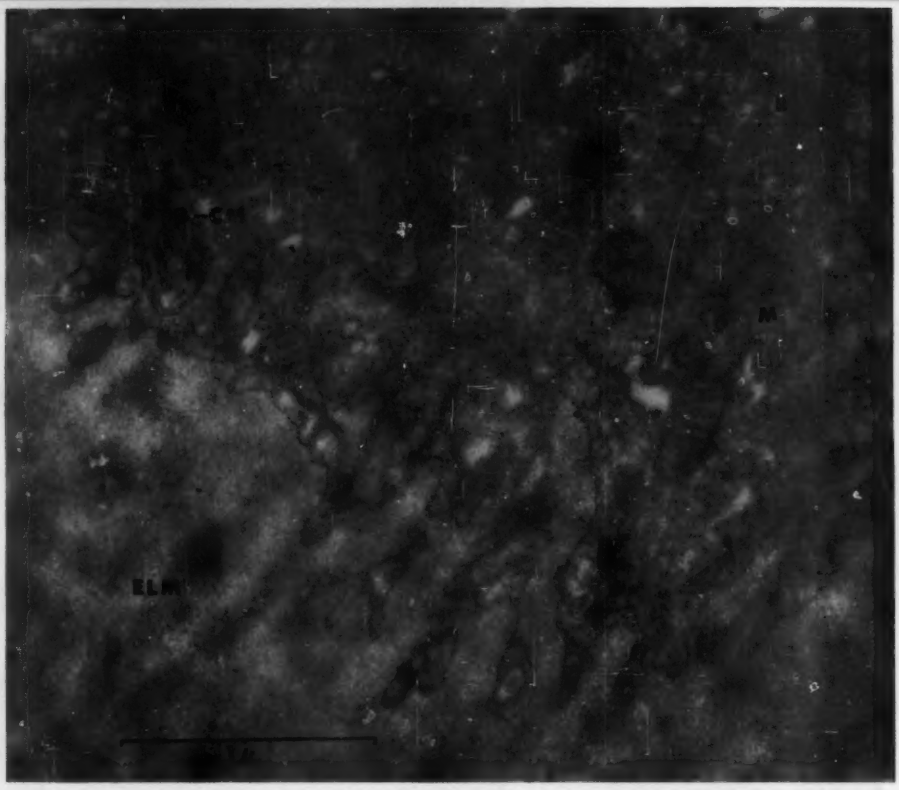


Fig. 10.—Basal part of a pigmented cell (*PE*) from a human eye. The external limiting membrane (*ELM*) is quite homogenous. The surface of the pigmented cell is very irregular. In the cytoplasm, some mitochondria (*M*) and β-cytomembrane (β-*LM*) are seen, and in the right upper corner, a border (*B*) to an adjacent pigmented cell is visible; $\times$ 50,000.

Fig. 11.—Middle and basal part of a nonpigmented cell (*NPE*) from a human eye. In the left lower corner of the picture a small part of pigmented cell (*PE*) is visible. There is a great amount of heavily stained granules in the nonpigmented cell. Note how the granules appear in aggregates. Magnification $\times$ 40,000.

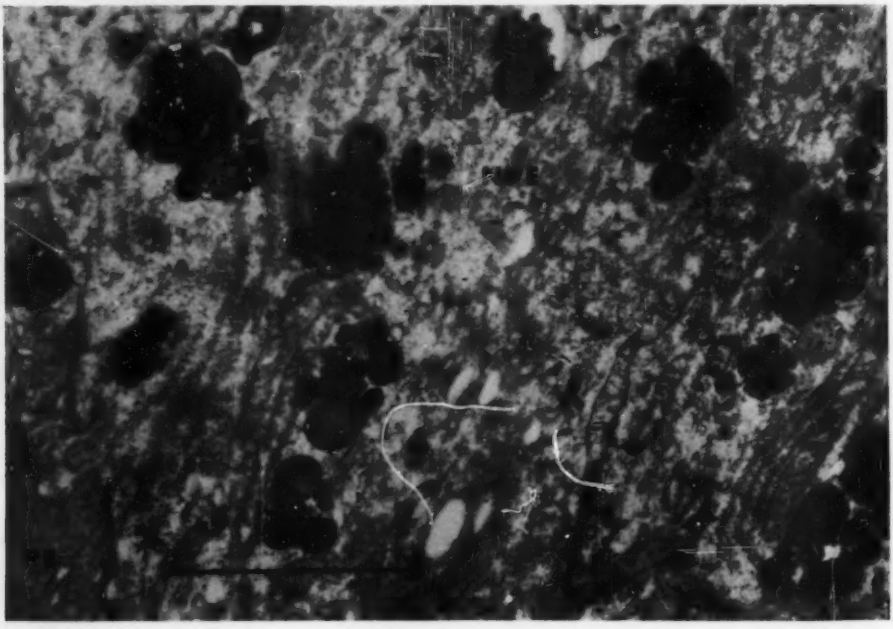


Fig. 1.—Survey picture of the outer part of the trabecular meshwork, Schlemm's canal (*SC*), and part of the sclera (*S*) from a human eye. The inner wall of Schlemm's canal consists of two endothelial linings, separated by a narrow space (*W*) in which one finds a homogenous material. In the lining next to Schlemm's canal there are large spaces (*SP*), which appear closed. Beneath the wall some endothelial cells with nuclei (*N*) are visible. Individual trabeculae (*T*) and intertrabecular spaces (*IS*) are also seen; × 8,200.

The figures on pp. 1047 to 1056 are for the article on pp. 956 to 958 by Holmberg.

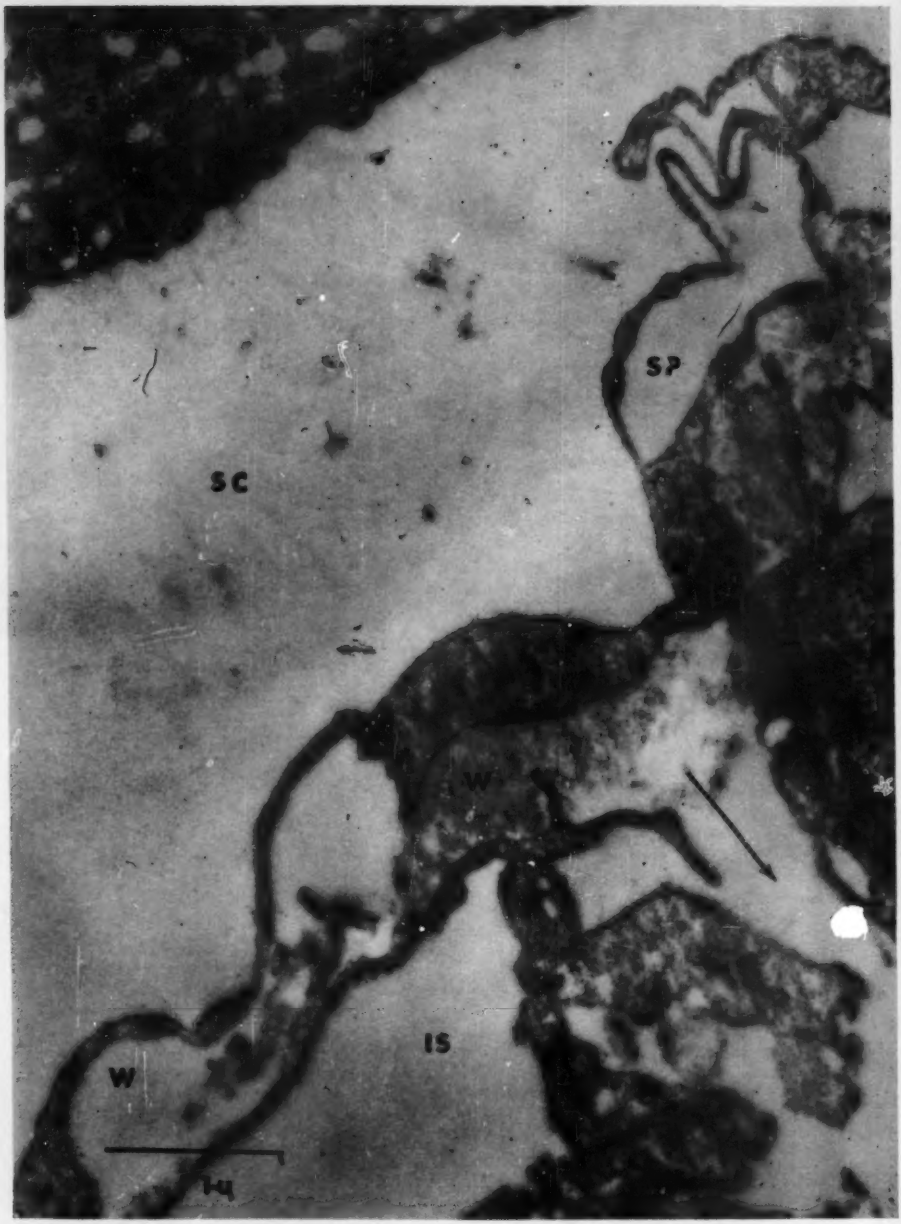


Fig. 2.—Part of the inner wall in higher magnification. The space (*W*) between the two endothelial linings has open communication (*arrow*) with the intertrabecular spaces (*IS*). *S*=sclera, *SC*=Schlemm's canal, *SP*=spaces in the endothelial lining next to Schlemm's canal. Human eye; × 27,000.

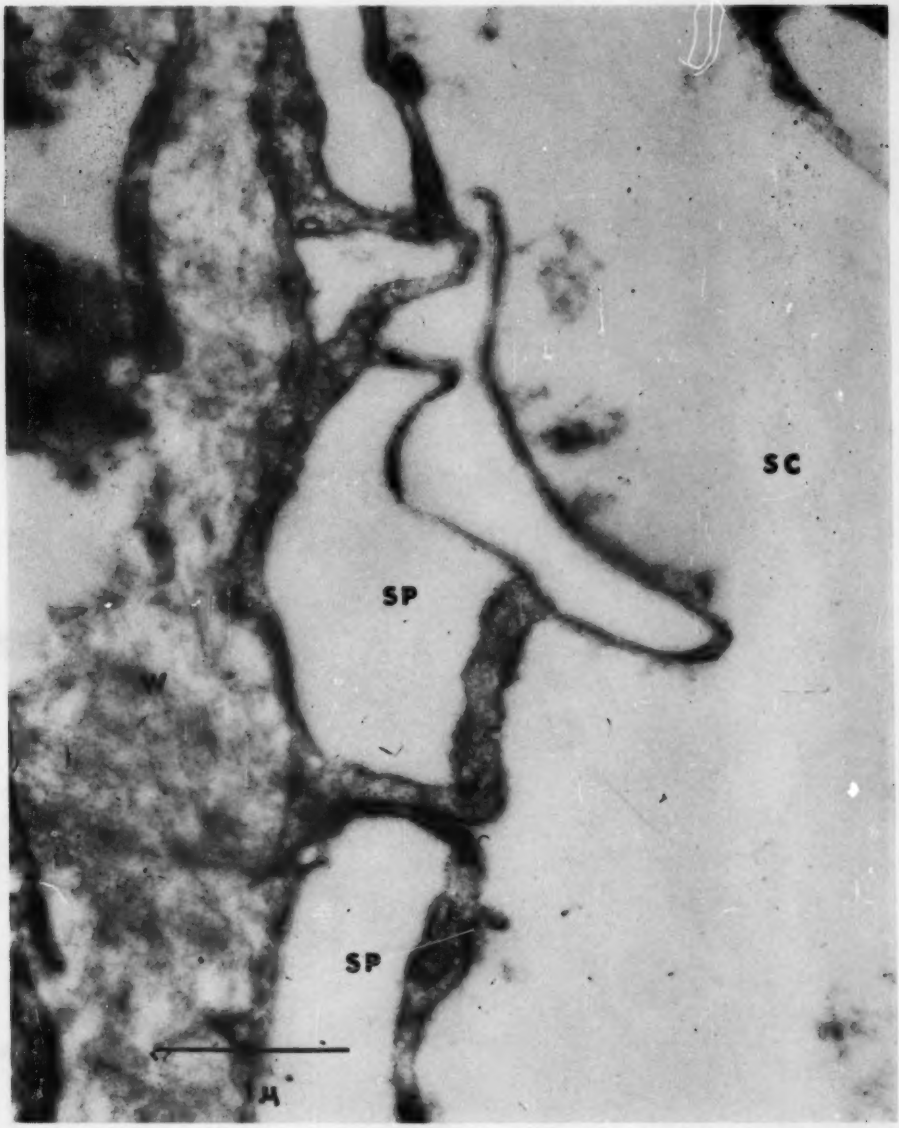


Fig. 3.—Several spaces (*SP*) in the endothelial lining next to Schlemm's canal. On this particular section they appear closed. *SC*=Schlemm's canal, *W*= the space between the two endothelial linings. Note the homogenous material present in this space. Human eye; × 30,000.

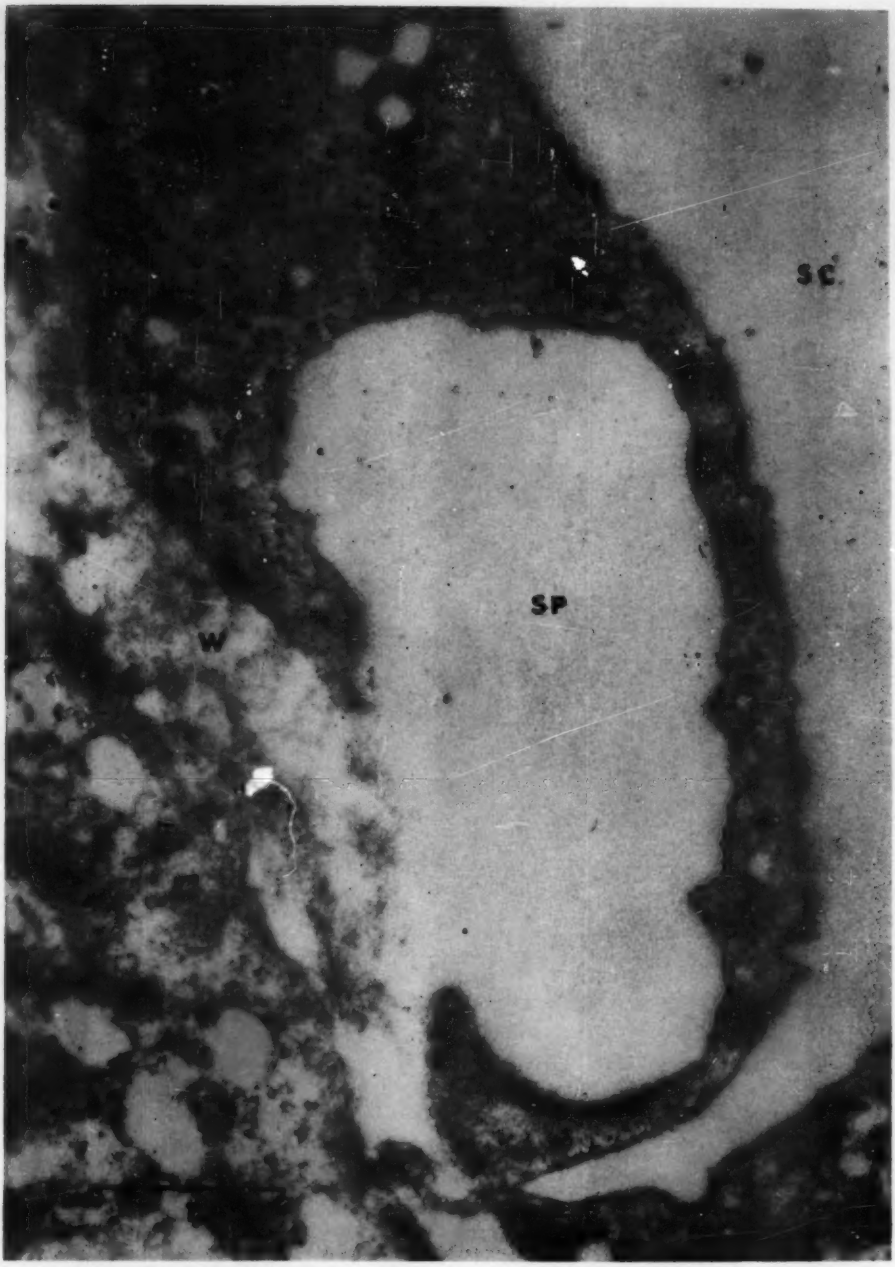


Fig. 4.—The space (*SP*) in the endothelial lining next to Schlemm's canal (*SC*) has open communication with the space (*W*) between the two linings which forms the inner wall of Schlemm's canal. Human eye; × 30,000.

Figs. 5-9.—Serial section through the endothelium (*N*=nucleus) next to Schlemm's canal (*SC*) with several spaces (in the previous figures indicated by *SP*) labeled *A*, *B*, *C* and *D*. Human eye; × 27,000.

Fig. 5.—All spaces appear closed.

Fig. 6.—*A*, *B*, and C communicate with each other.

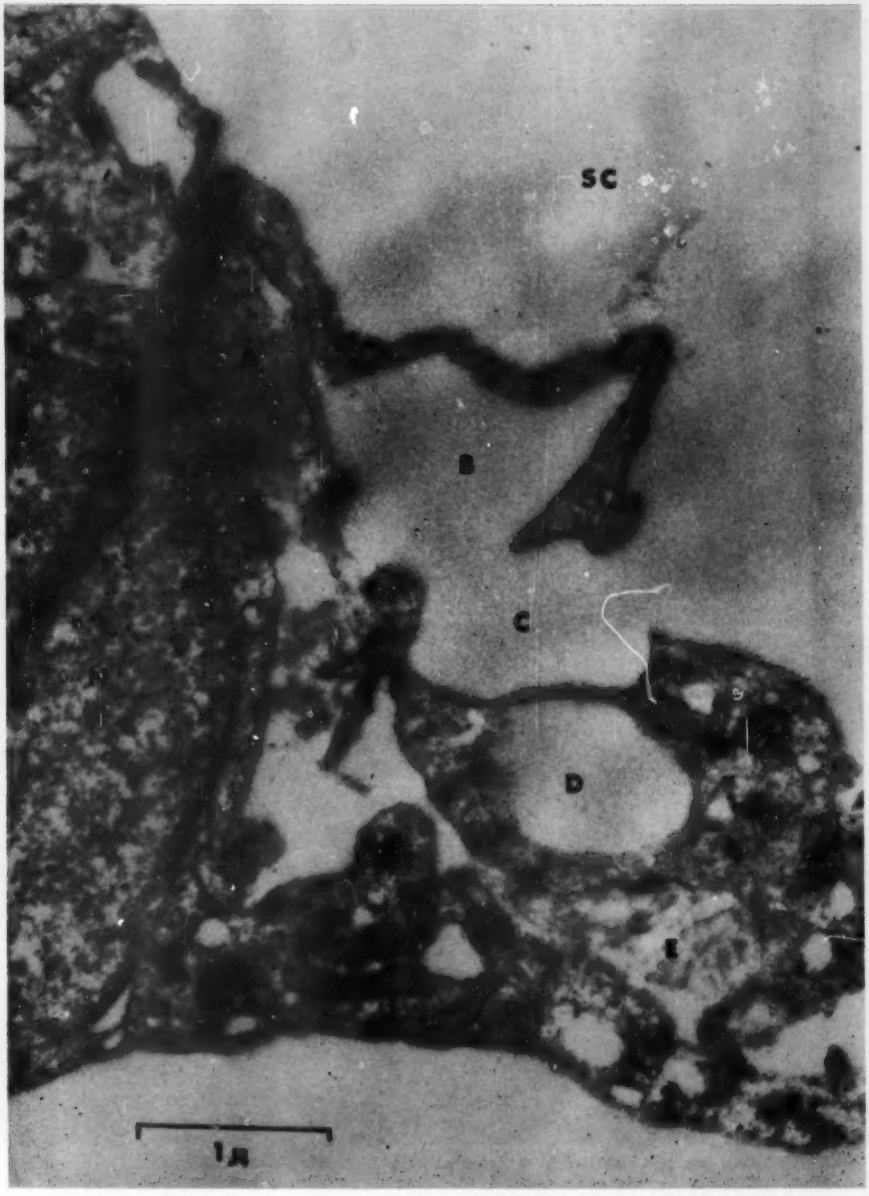


Fig. 7.—*B* and *C* have free communication with Schlemm's canal. In this figure another space *E* has been visible.

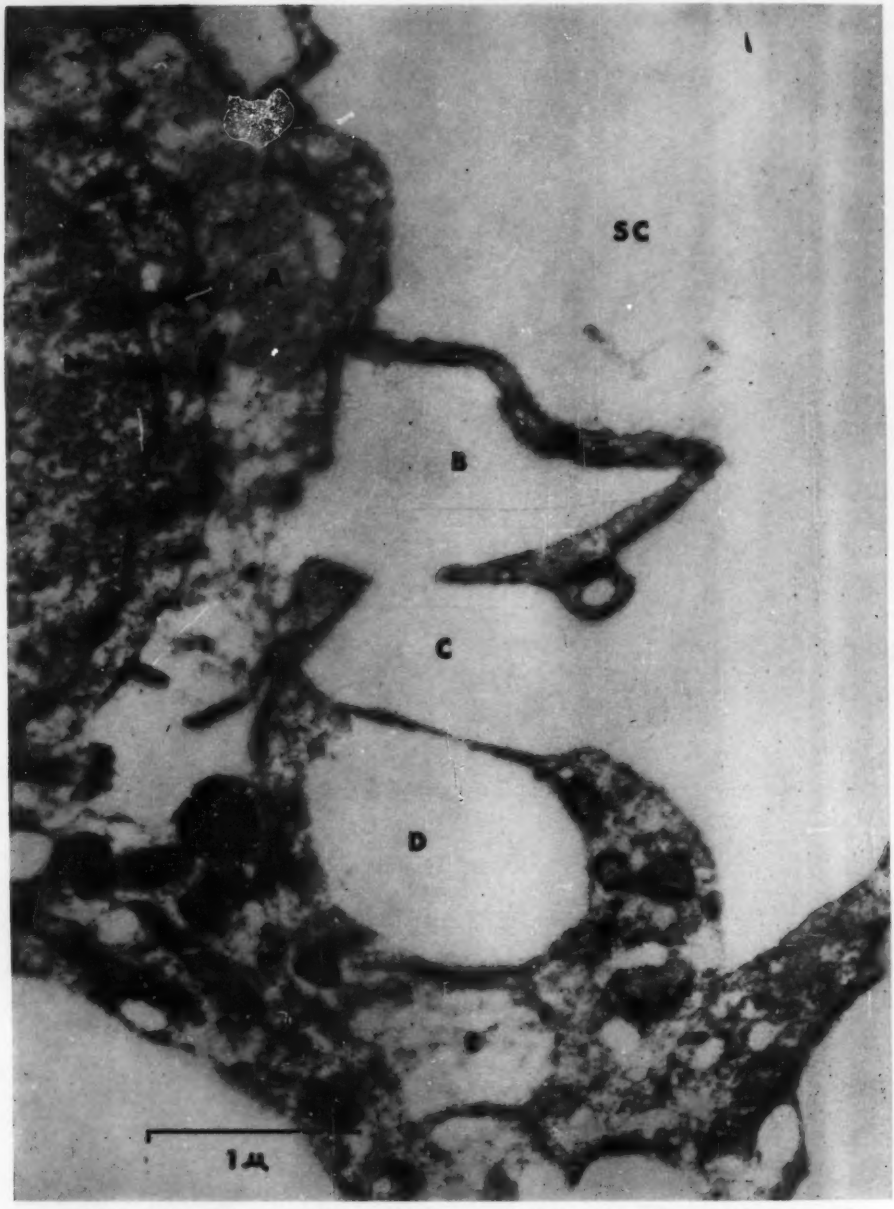


Fig. 8.—*B* has still an opening to Schlemm's canal. *C* has almost disappeared. *D* is much larger and closer to Schlemm's canal.

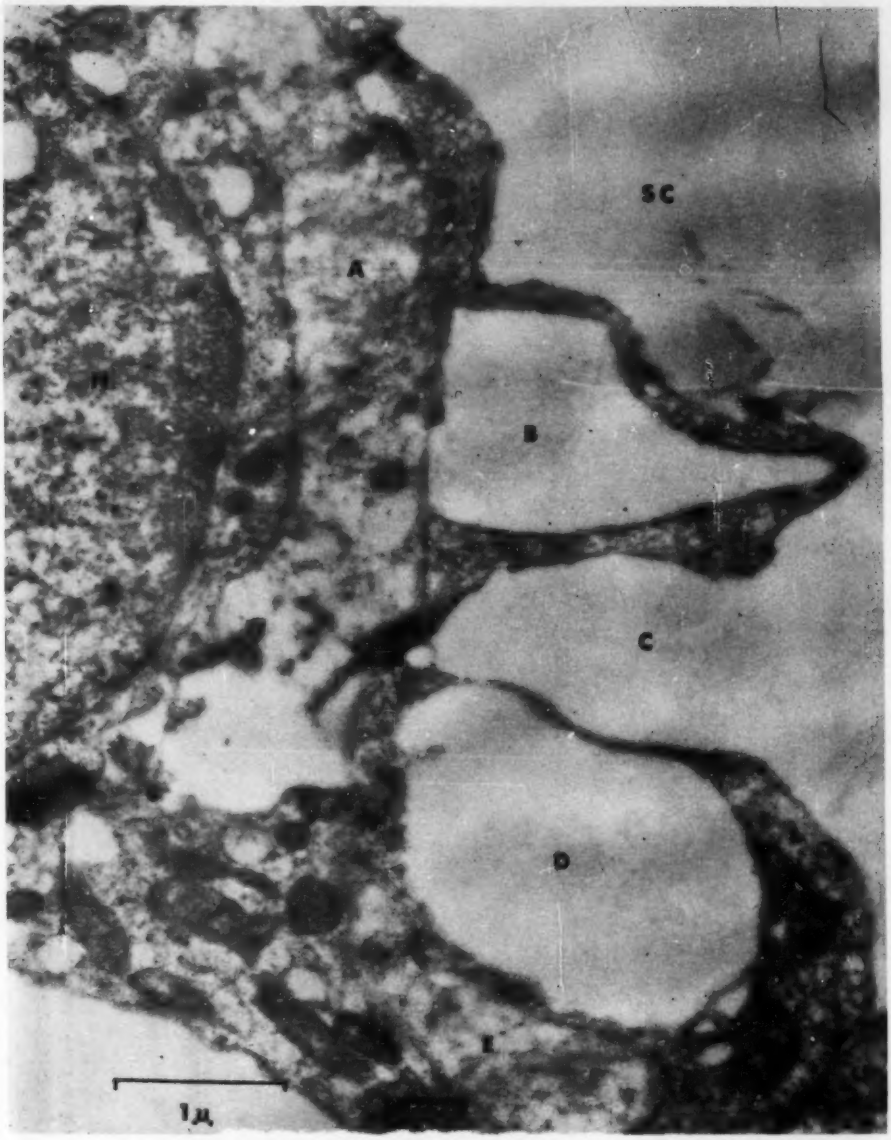


Fig. 9.—*A* has still communication with *B*. *B* is closed and *C* has completely disappeared. Reduced 11% of original.

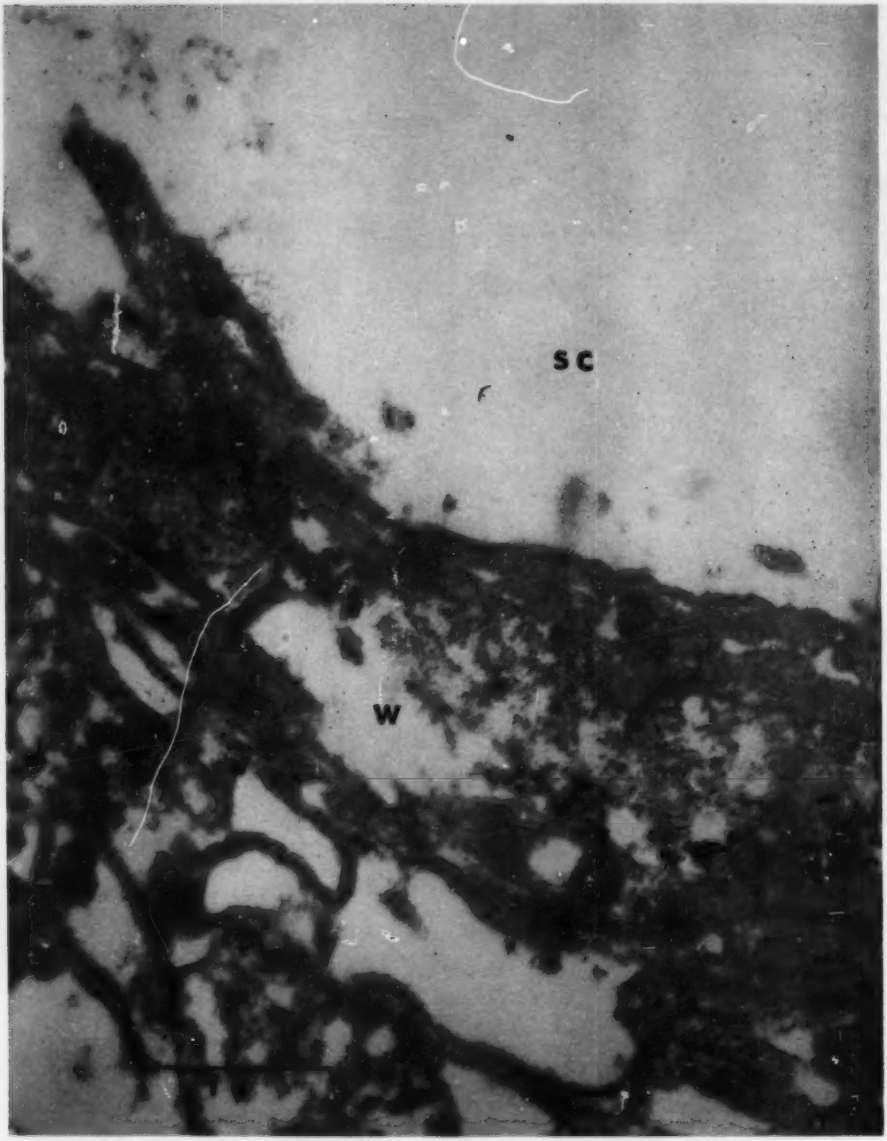


Fig. 10.—The outermost part of the trabecular meshwork from a monkey eye injected with saccharated iron 15 minutes before fixation. The inner wall is built in the same way as in human eye, with two endothelial linings separated by a narrow space (*W*). The iron particles (black dots) are localized only within areas where the homogenous material (gray background) is present. *SC*=Schlemm's canal; × 30,000.

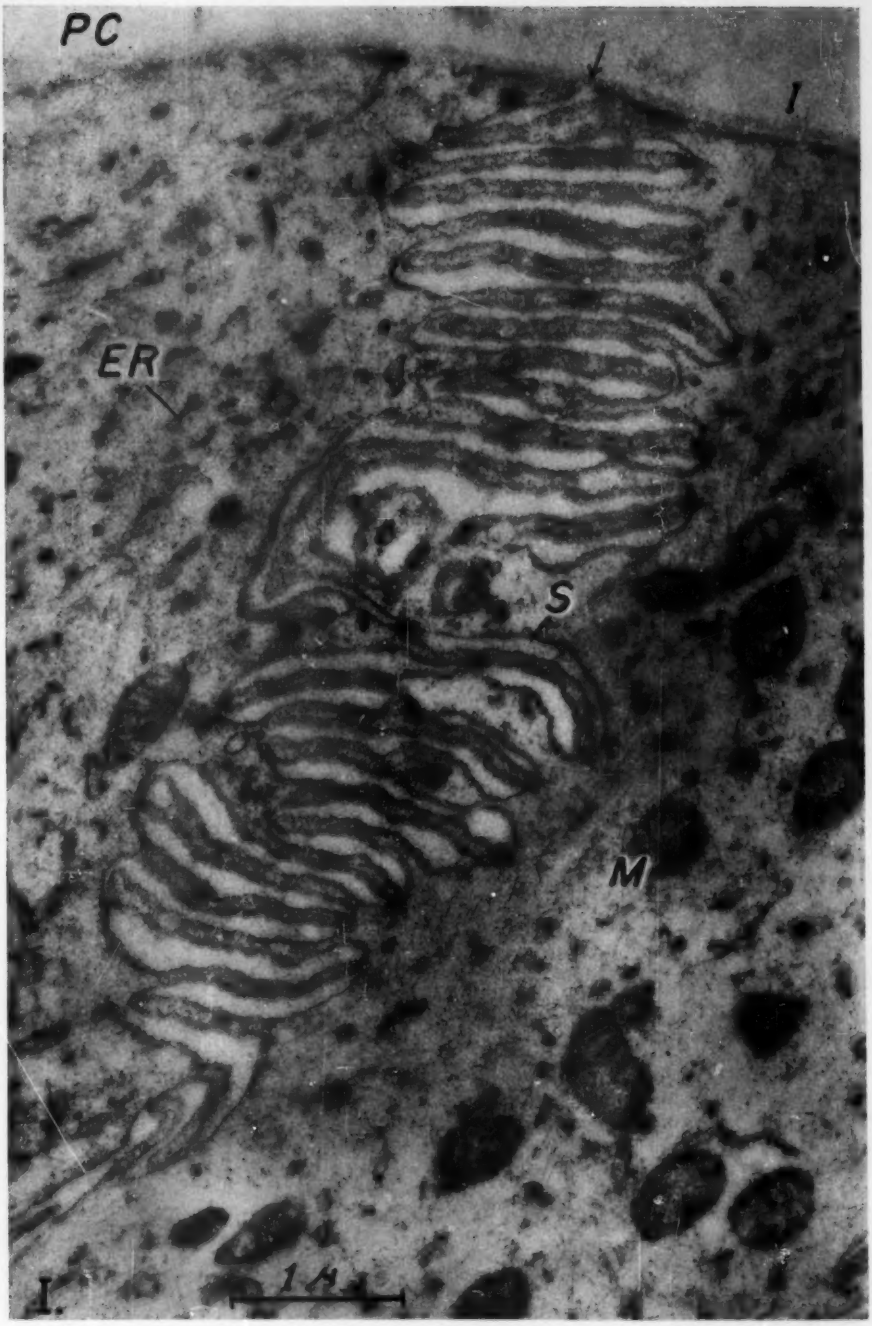


Fig. 1.—Section of the ciliary processes of a normal adult albino rabbit. The margin (at arrow) between the ciliary epithelial cells which face the posterior chamber (*PC*) is characterized by elaborate interdigitations of their limiting membranes. The amount of intercellular space (*S*) between these elaborated membranes varies greatly. The free surface of these cells is lined by the internal limiting membrane (*I*). Elements of the endoplasmic reticulum (*ER*) as well as mitochondria (*M*) are found in the cytoplasm; × 26,500.

The figures on pp. 1057 to 1066 are for the article on pp. 959 to 965 by Pappas et al.

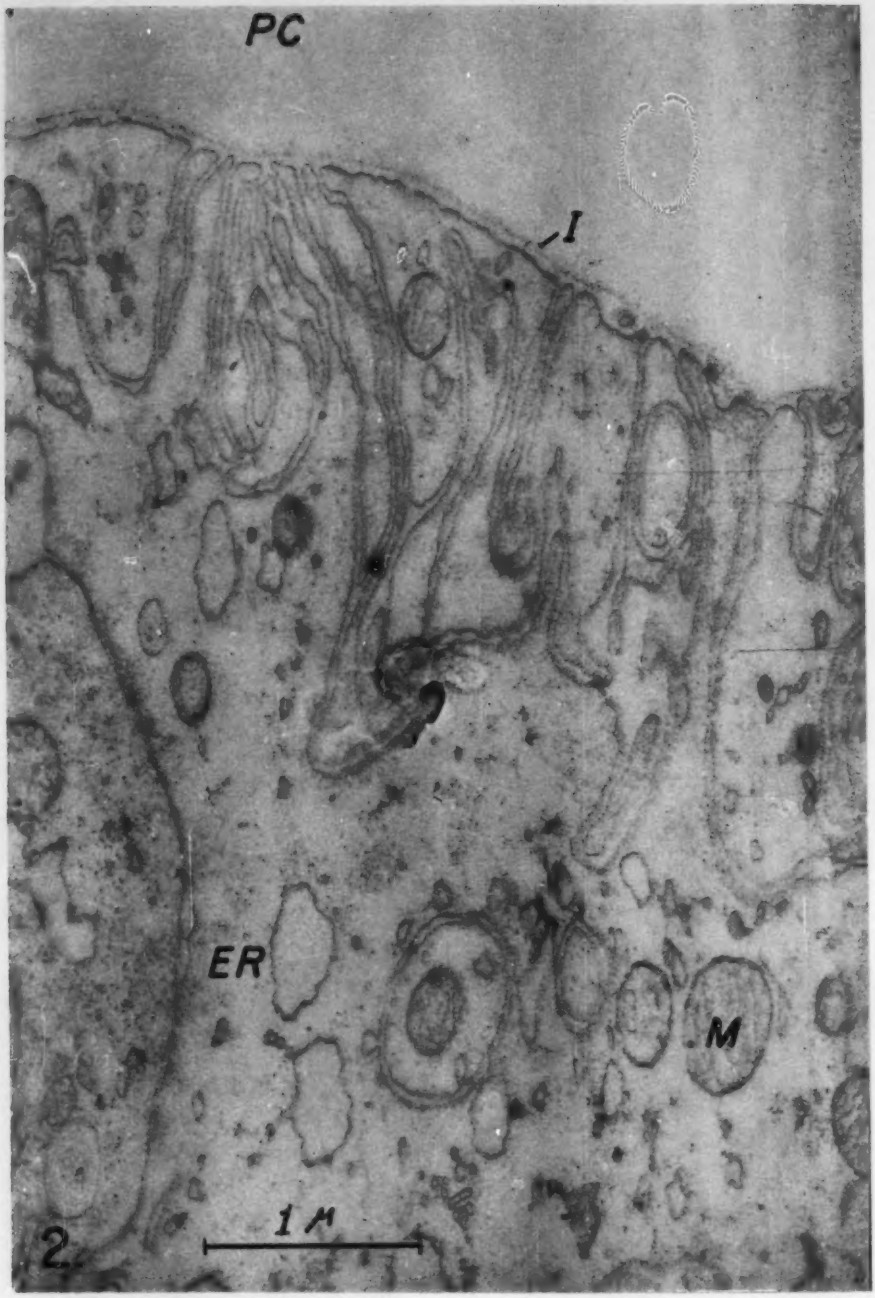


Fig. 2.—Portion of an epithelial cell from a ciliary process of a normal adult rabbit. The surface of the cell facing the posterior chamber (*PC*) is characterized by a large number of infoldings of the cell membrane. The internal limiting membrane (*I*) lines the free surface of these cells. Vesicular elements of the endoplasmic reticulum (*ER*) and mitochondria (*M*) are present; × 34,000.

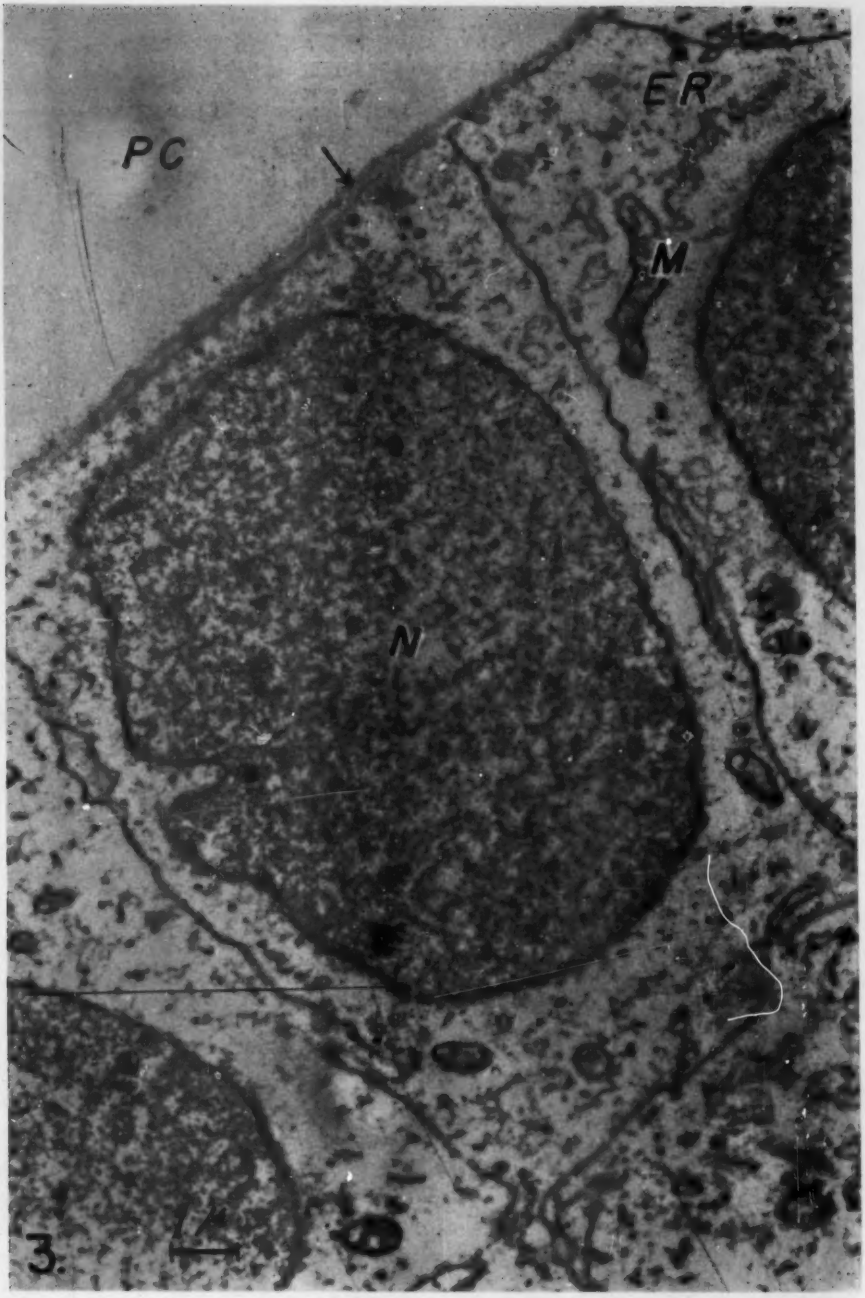


Fig. 3.—Section of ciliary epithelium taken from newborn albino rabbit. Unlike the cells of the adult ciliary epithelium (Figures 1 and 2), these cells lack the elaborate interdigitated margins as well as the infoldings of the surface membrane facing the posterior chamber (*PC*). The internal limiting membrane lining the free surface of these cells can be seen at arrow. Cytoplasmic constituents, such as the nucleus (*N*), mitochondria (*M*) and endoplasmic reticulum (*ER*), are present; × 11,000.

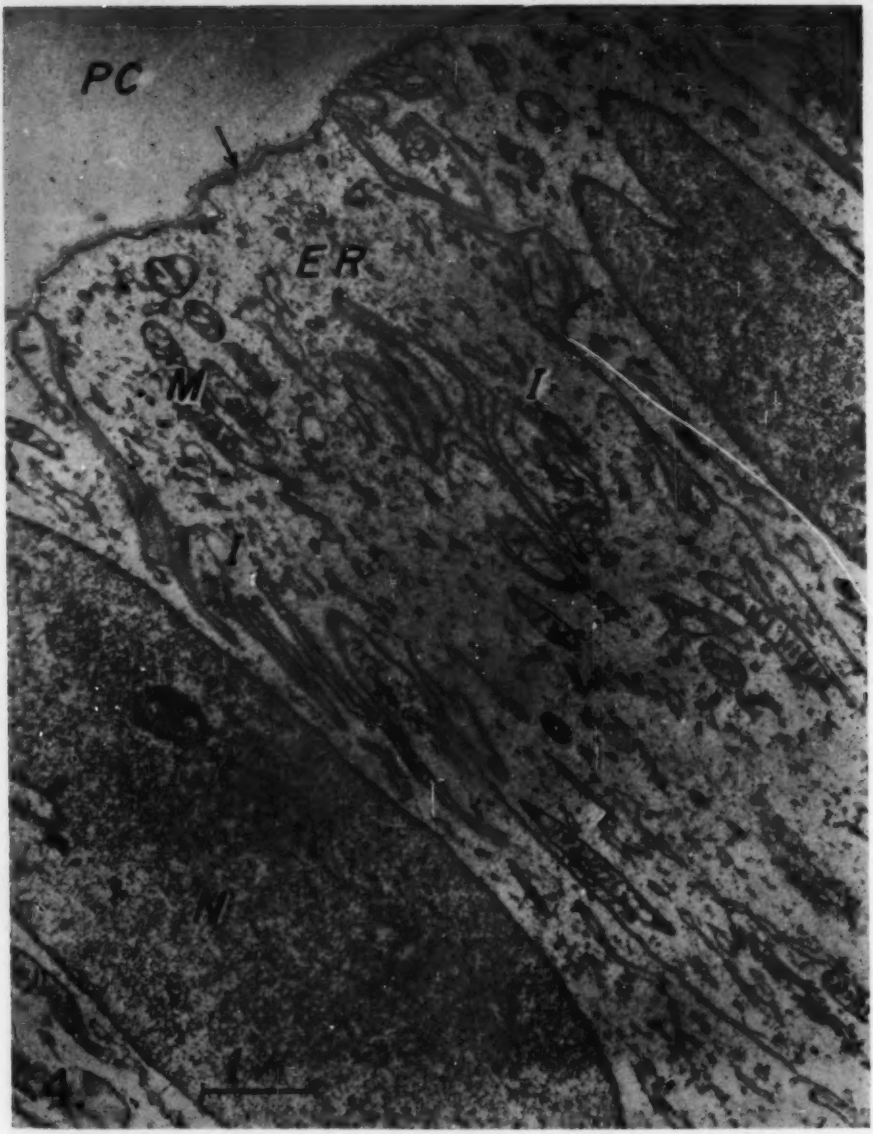


Fig. 4.—Ciliary epithelium of a 7-day-old albino rabbit, showing the cells which face the posterior chamber. The interdigitated margins which characterize the cells of the adult are first becoming evident. The onset of aqueous secretion coincides with the appearance of these interdigitations. Only a very few surface infoldings are found at this time. Nucleus (*N*), mitochondria (*M*), endoplasmic reticulum (*ER*), and the internal limiting membrane (at arrow) can be seen; × 18,000.

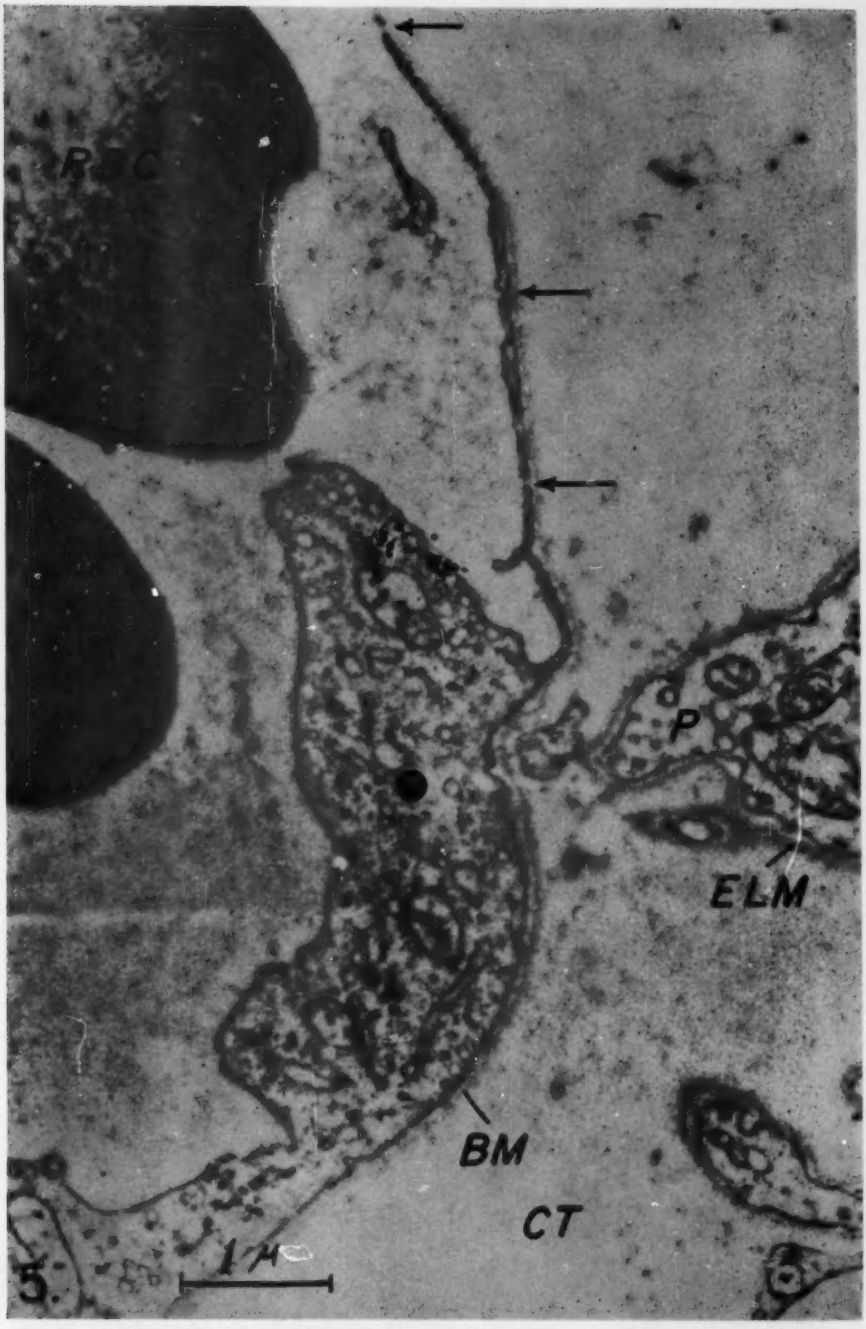


Fig. 5.—A portion of a capillary from a ciliary process of an adult albino rabbit. The endothelial cell boundary is very thin at the upper half of the micrograph. Note pores at arrows. The diameter of the pores is about 400 Å. A basement membrane (*BM*) lines the surface of the endothelial cell which faces the connective tissue stroma (*CT*). Two erythrocytes (*RBC*) are found in the lumen of the capillary. The external limiting membrane (*ELM*) lines the basal portion of the pigment cell (*P*); × 24,000.

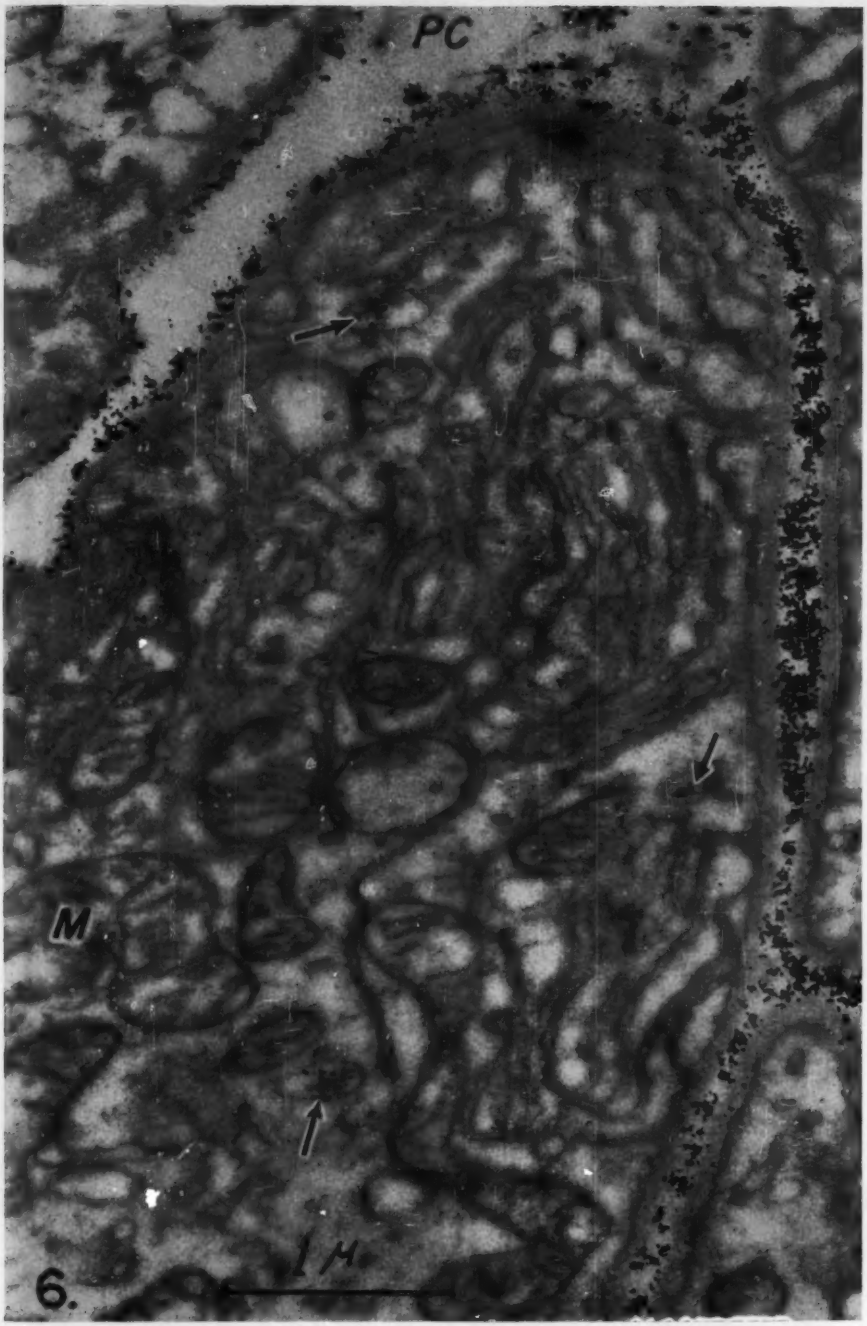
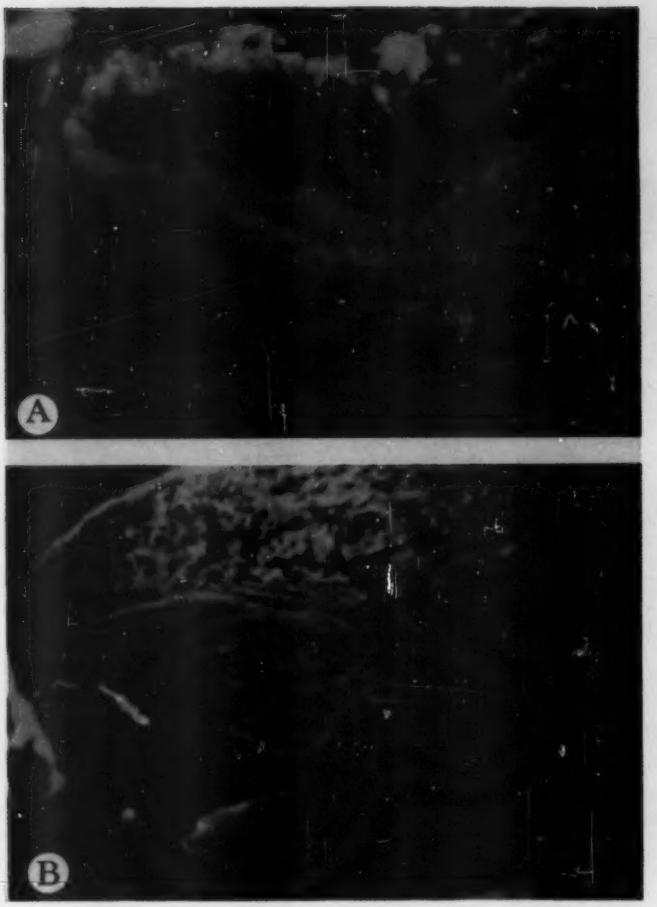


Fig. 6.—Adult ciliary epithelial cells fixed 20 minutes after thorium dioxide was injected into the posterior chamber (*PC*). Thorium particles adhere to the internal limiting membrane lining the free surface of the cells. The infoldings contain thorium particles. It is evident that some reabsorption occurs in cell surface infoldings. Note that numerous vesicles in the cytoplasm also contain thorium (at arrows). *M* indicates mitochondria; × 36,000.



Sections (2μ thick) of frozen dried ciliary epithelium photographed with the Zeiss fluorescence microscope. The exciter filters used were the BG-12 and Corning No. 7-59. The ocular filter was the OG-5. This combination permitted a sharp color difference between areas where the fluorescein concentration was high (yellow) and areas where it was low and therefore masked by the blue tissue autofluorescence. The plate was recorded on Super Anscochrome (daylight type) at an exposure of 45 seconds, and an original magnification of $\times$ 500. *A*, a fluorescence micrograph of normal adult ciliary epithelium which was frozen dried 40 minutes after the systemic injection of sodium fluoresceinate. Some fluorescein was visible in the aqueous at the time of enucleation. Yellow fluorescein can be seen adherent to the zonular fibers (above). It also appears as caps on the cells facing the posterior chamber in the area of the surface infoldings, and in the area corresponding to the division between the two cell layers. *B*, a fluorescence micrograph of the ciliary epithelium taken from an eye with an open fistula at the limbus. Systemic injection of the sodium fluoresceinate was followed by the almost immediate appearance of fluorescein in the aqueous. The eye was enucleated 5 minutes after the injection of sodium fluoresceinate. The fluorescein appears to be in the basement membrane area, and in the area between cells which corresponds to the interdigitations. Negligible fluorescein appears in the region of the apical infoldings facing the posterior chamber.

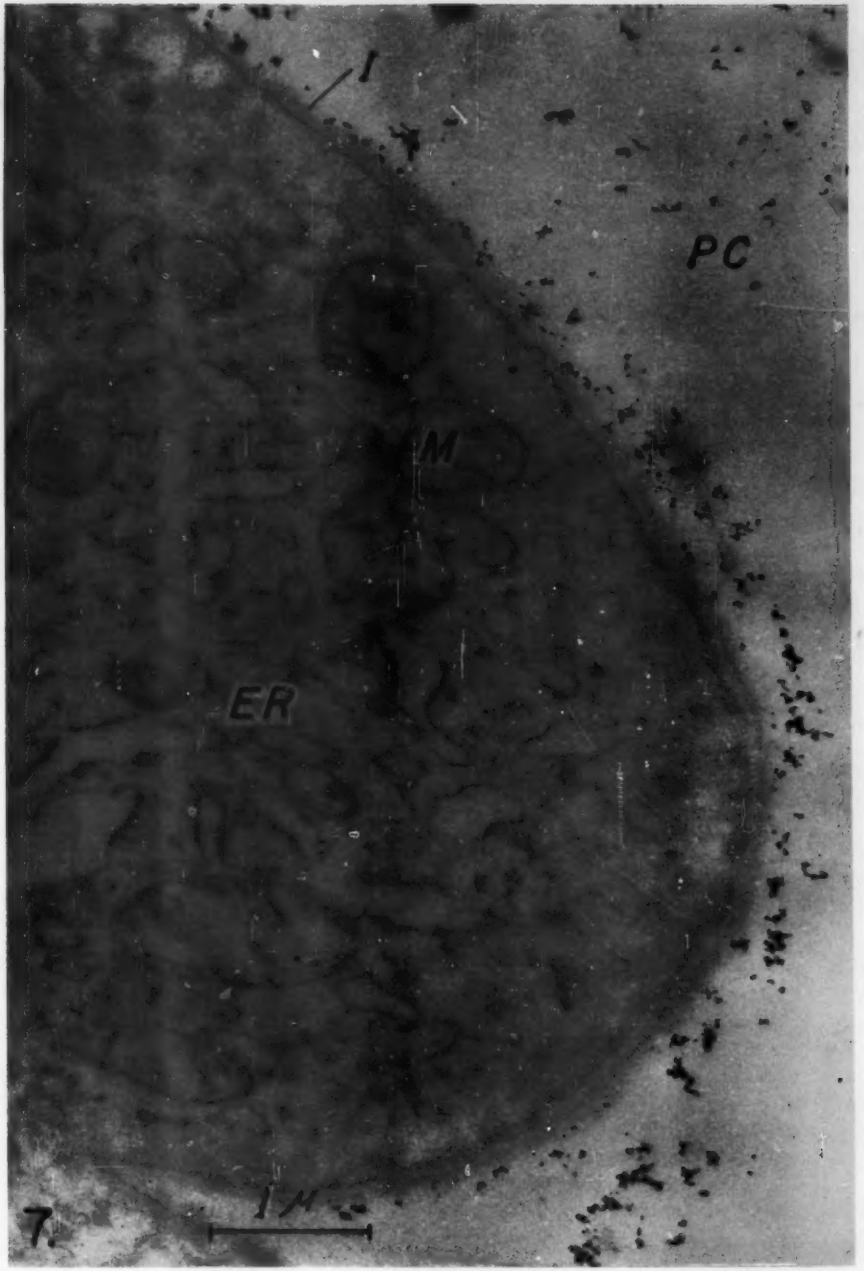


Fig. 7.—Ciliary epithelium taken from adult rabbit three to four hours after a systemic injection of Shigella endotoxin. The aqueous humor appeared cloudy, indicating the presence of plasma proteins. It has been shown that under such circumstances the membrane elaborations of the cells facing the posterior chamber (*PC*) tend to disappear (Pappas and Smelser). Twenty minutes before fixation, thorium dioxide was injected into the posterior chamber. Unlike in the normal eye (Figure 6), the thorium adheres very poorly to the internal limiting membrane (*I*) lining the free surface of these cells. The mitochondria (*M*) as well as elements of the endoplasmic reticulum (*ER*) are swollen; × 24,500.

Fig. 8.—As in Figure 6, the ciliary epithelium shown in this electron micrograph was taken from an adult rabbit after systemic injection of endotoxin. However, in this case, the aqueous humor was clear, indicating that plasma proteins had not yet leaked through to a significant extent. Most of the infoldings are still present. The thorium particles, injected into the posterior chamber (*PC*), 20 minutes before fixation, do not adhere very well to the internal limiting membrane (*I*) lining the free surface of these cells. It is important to note that, although infoldings are still present, no thorium is found in them nor are vesicles containing thorium found in the cytoplasm. It appears likely that the remaining infoldings have lost their ability to reabsorb materials from the aqueous. *M* indicates mitochondria; × 42,000.

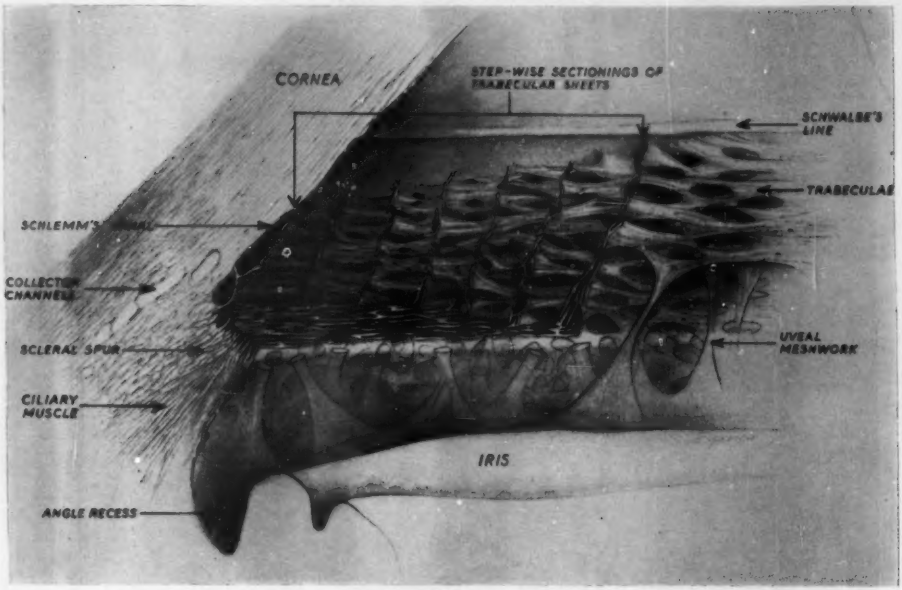
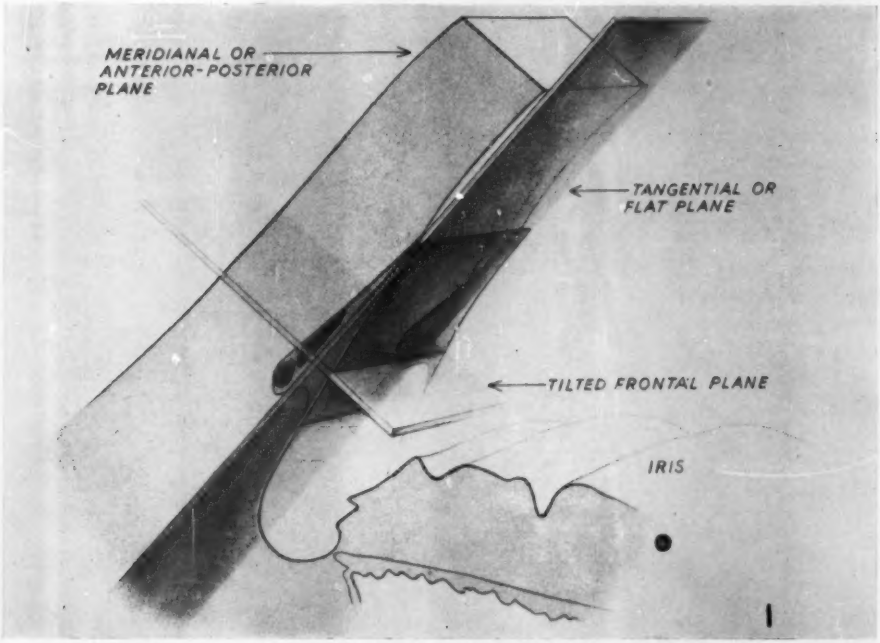


Fig. 1.—Diagram of trabeculae showing stepwise dissection of the trabecular sheets from anterior chamber to Schlemm's canal.

Fig. 2.—Diagram showing the three planes of sectioning commonly used in studying trabeculae.



The figures on pp. 1067 to 1076 are for the article on pp. 966 to 973 by Garron and Feeny.

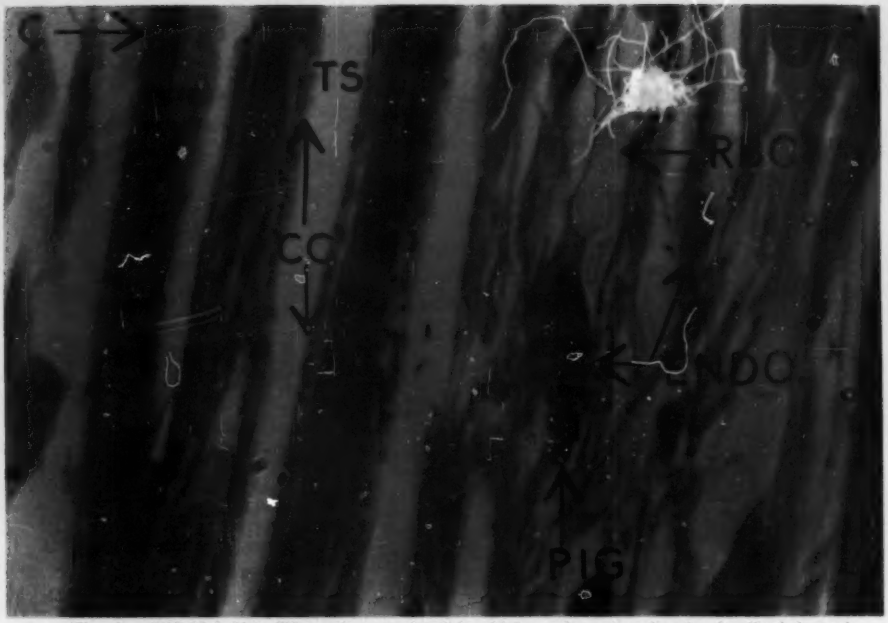


Fig. 3.—(707-N-2-134) Photomicrograph of 1μ thick section of trabeculae in tilted frontal plane showing: *C*, collagen core; *TS*, intertrabecular space; *RBC*, red blood cells; *PIG*, pigment clumps; *ENDO*, endothelial nuclei; *CC*, granules, previously called "elastic fibers"; $\times$ 1400; reduced 14% of the original.

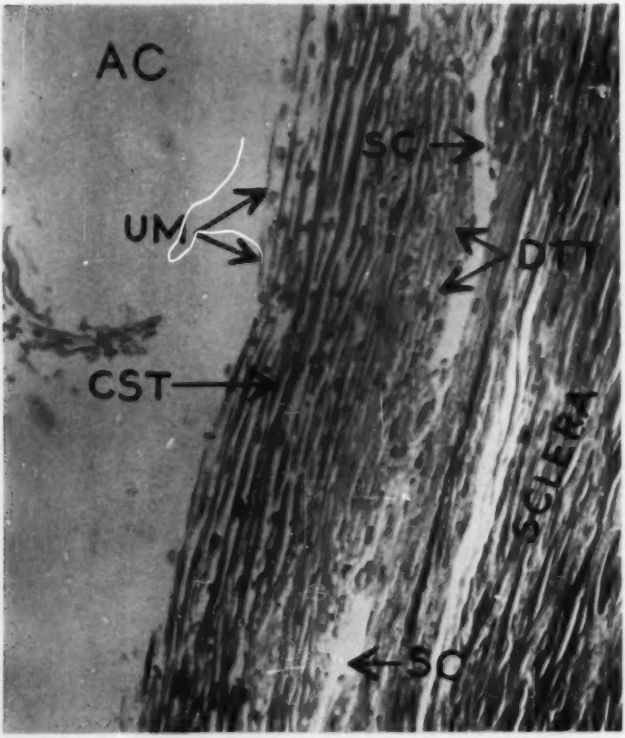


Fig. 4.—Lower power of Figure 3. Collagen core diminishes in thickness as Schlemm's canal approached. *CST*, corneoscleral trabeculae; *DTT*, pore tissue adjacent to Schlemm's canal; *SC*, Schlemm's canal; *UM*, uveal fibers; $\times$ 175; reduced 12% of the original.

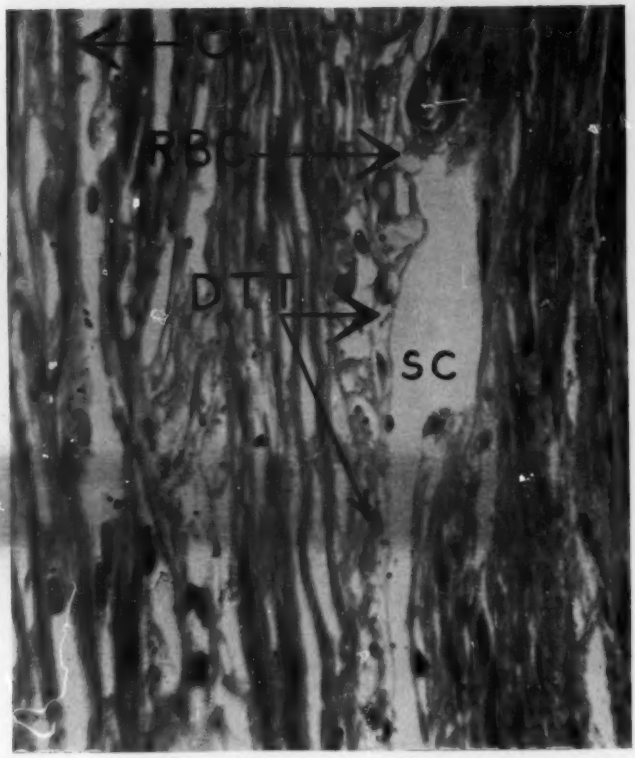
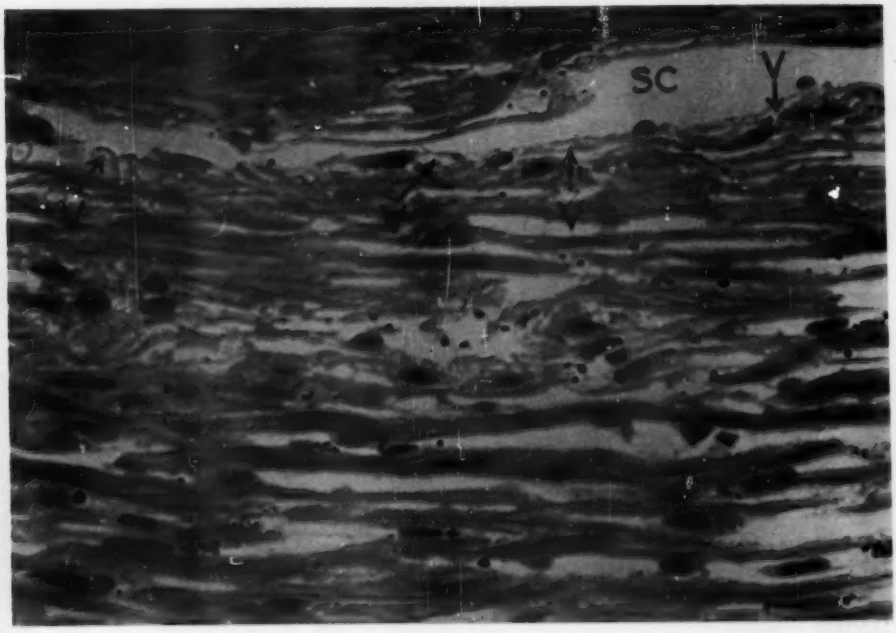


Fig. 5.—(707-N-2-134) Photomicrograph of 1μ thick section of trabecular tissue in tilted frontal plane. *C*, collagen core; *DTT*, pore tissue of varying thicknesses; *SC*, Schlemm's canal; *RBC*, red blood cell trapped in opening from Schlemm's canal; × 560; reduced 12% of the original.

Fig. 6.—(707-N-2-132) Photomicrograph of 1μ thick section of trabecular tissue in tilted frontal plane showing "vacuolated" cells on inner wall of canal (*V*). *SC*, Schlemm's canal; reduced 14% of the original.



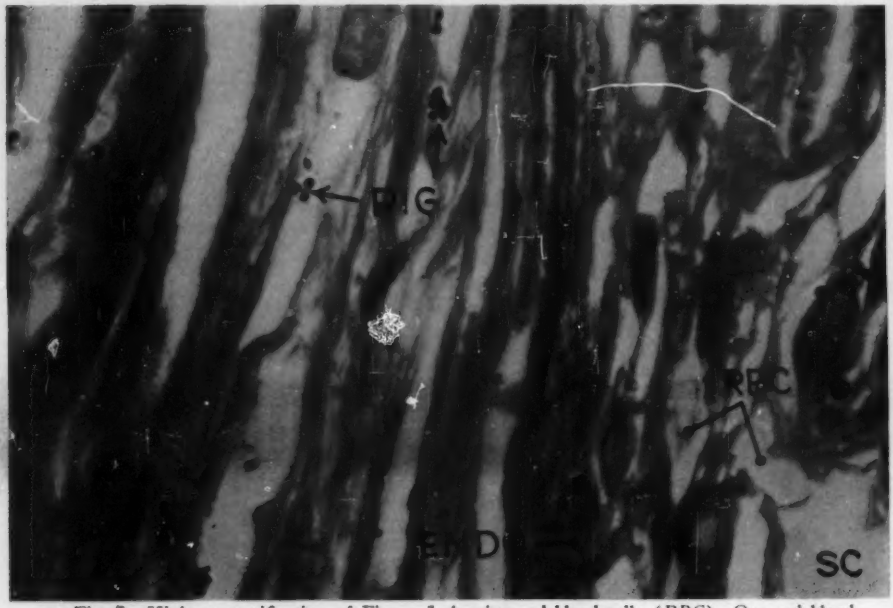
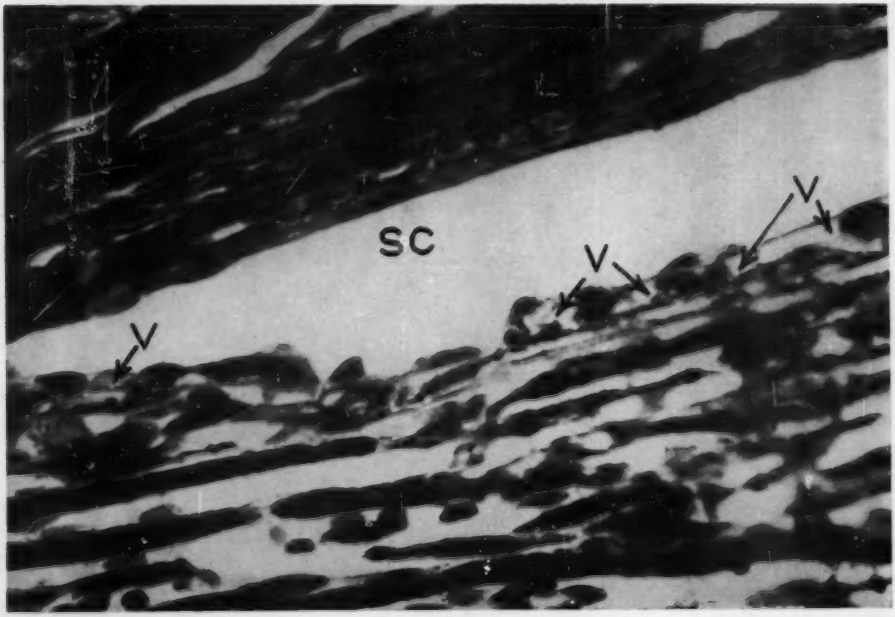


Fig. 7.—Higher magnification of Figure 5 showing red blood cells (*RBC*). One red blood cell is trapped in an opening from Schlemm's canal while another is seen in an intertrabecular space. *PIG*, pigment granules; *SC*, Schlemm's canal; *ENDO*, endothelial nucleus; × 1,400; reduced 14% of the original.

Fig. 8.—(58-34) Photomicrograph of trabecular tissue sectioned in the meridional plane. Note "vacuoles" on inner wall of canal (*V*); reduced 14% of the original.



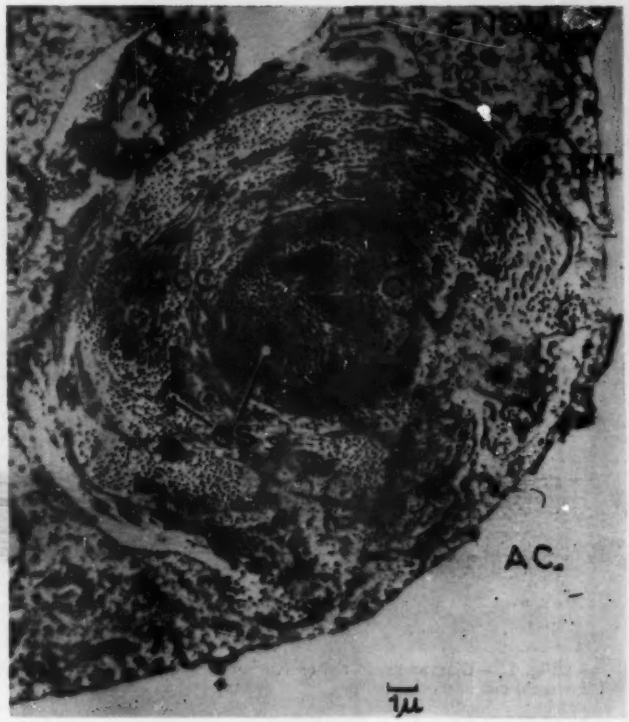
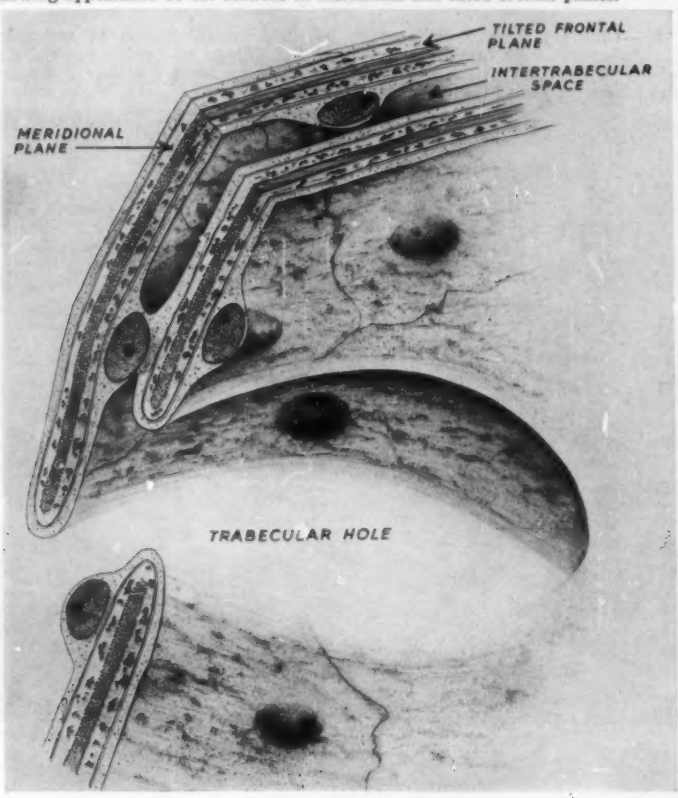


Fig. 9.—(857-18A) Electron micrograph of uveal fiber. *C*, collagen fibers cut in cross section; *CS*, amorphous cement substance; *CC*, irregular clumps of 1,000 A.-banded material; *ENDO*, endothelium; *BM*, basement membrane. Section was treated with uranium acetate; × 5,000; reduced 14% of the original.

Fig. 10.—Diagrammatic representation of portions of two adjacent corneoscleral trabecular sheets showing appearance of cut sections in meridional and tilted frontal planes.



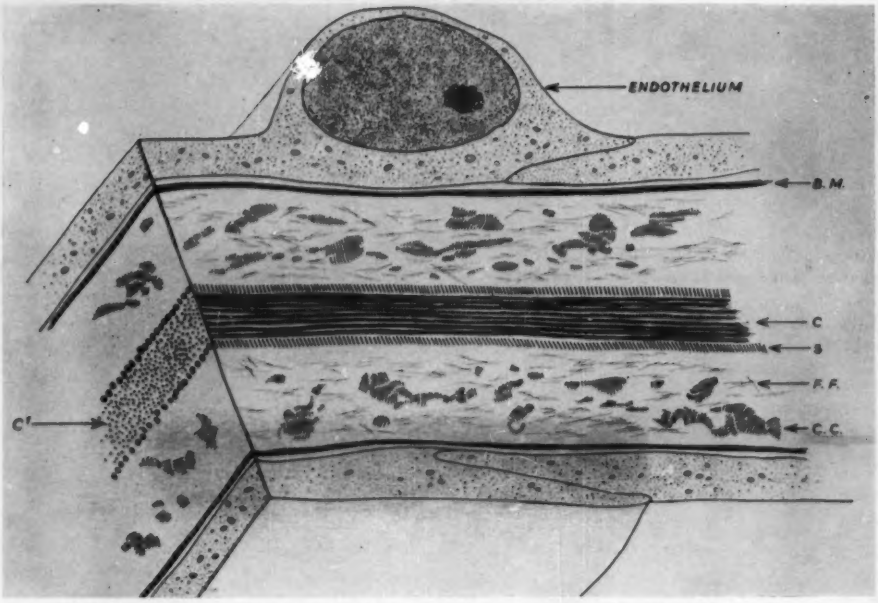
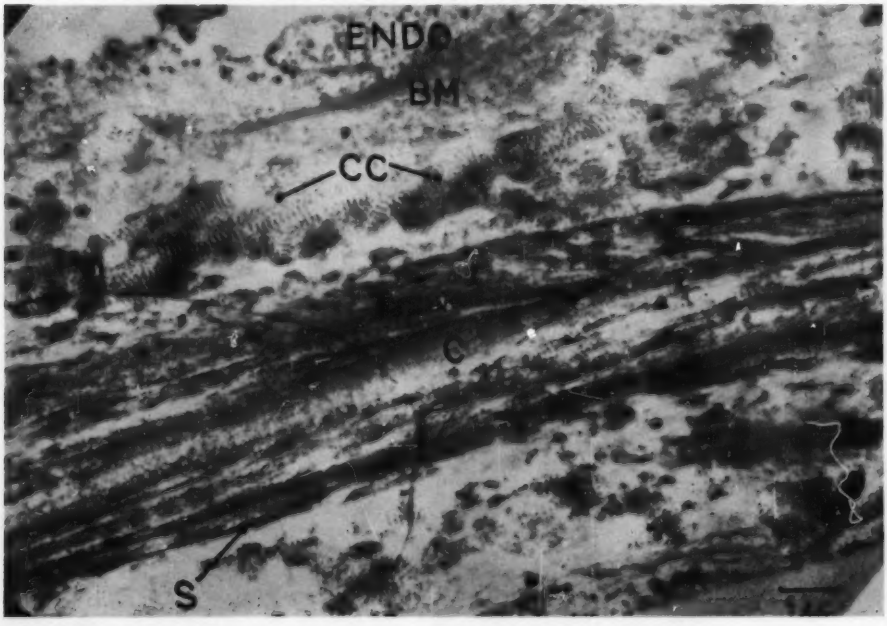


Fig. 11.—Diagrammatic representation of the electron microscopic appearance of a trabecular sheet cut in meridional and tilted frontal planes. *BM*, basement membrane; *C*, collagen core cut longitudinally in tilted frontal section; *S*, sheath of 1,000 A.-banded fibers; *FF*, fine fibers in homogeneous matrix; *CC*, clumped material showing 1,000 A. periodicity. Collagen fibers, *C'*, are cut in cross section when the trabeculae are sectioned in the meridional plane.

Fig. 12.—(610-3D) Electron micrograph of tilted frontal section of trabecular sheet. *ENDO*, endothelium; *BM*, basement membrane; *CC*, clumps of banded material with 1,000 A. periodicity; *C*, regular collagen cut longitudinally showing banding of approximately 640 A.; *S*, sheath of fibers showing 1,000 A. banding; × 11,000; reduced 14% of the original.



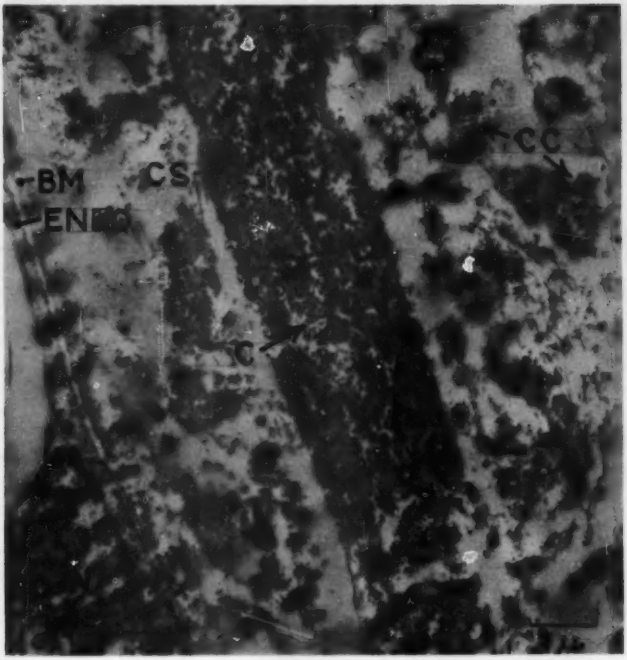


Fig. 13.—(814-22B) Electron micrograph of meridional section of trabecular sheet. *C*, collagen fibers cut in cross section; *CS*, cement substance around collagen fibers; *CC*, clumps of 1,000 A.-banded material in homogeneous matrix; *ENDO*, endothelium; *BM*, basement membrane; *N*, nucleus; × 12,000; reduced 14% of the original.

Fig. 14.—(610-4D) Electron micrograph of a tilted frontal section of trabecular sheet as it tapers down toward trabecular opening. *CM*, cell membrane; *C*, collagen fibers cut somewhat obliquely as they diverge above or below opening; × 11,000; reduced 16% of the original.

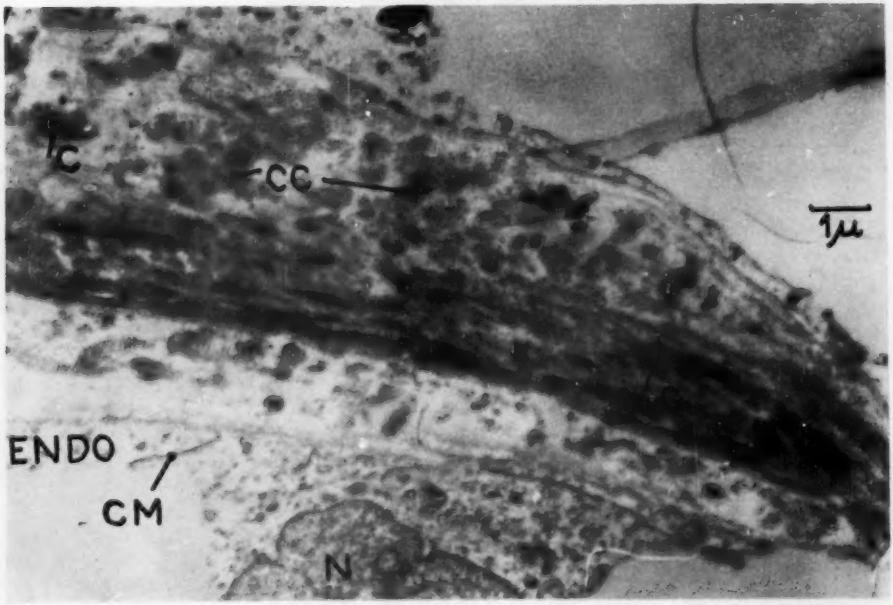


Fig. 15.—(610-5D) Electron micrograph of an oblique section of a trabecular sheet showing components of homogeneous matrix. *HM*, homogeneous matrix; *FF*, fine fibers; *CC*, clumped material with 1,000 A. periodicity; *C*, regular collagen (640 A.); *S*, sheath with 1,000 A. periodicity; × 11,400; reduced 14% of the original.

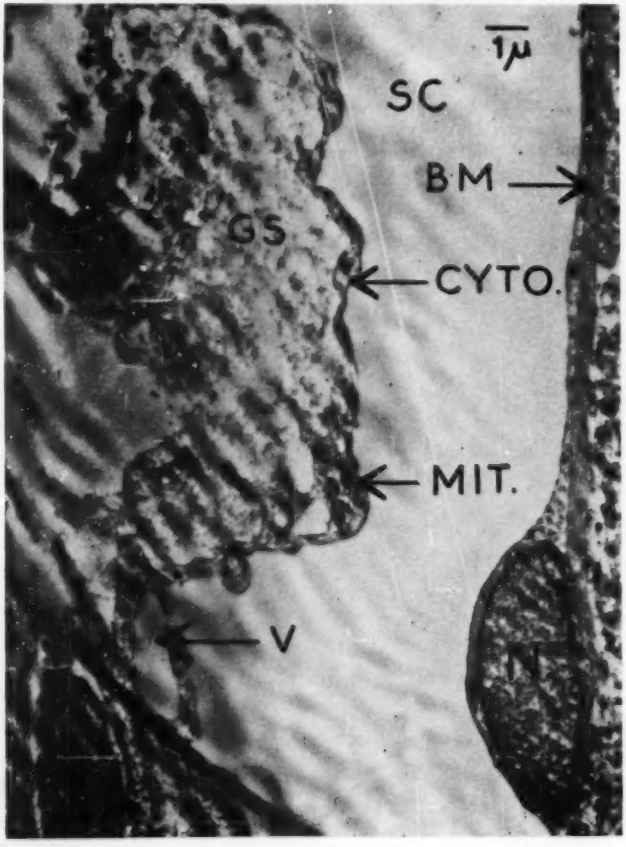


Fig. 16.—(754-3B) Electron micrograph showing internal and external walls of Schlemm's canal. Note thick basement membrane (*BM*) on the scleral side and its absence on trabecular side. *SC*, Schlemm's canal; *GS*, ground substance; *CYTO*, endothelial cytoplasm; *MIT*, mitochondria; *V*, "vacuolization" of endothelial cytoplasm; *N*, nucleus of endothelial cell; × 7,500; reduced 12% of the original.

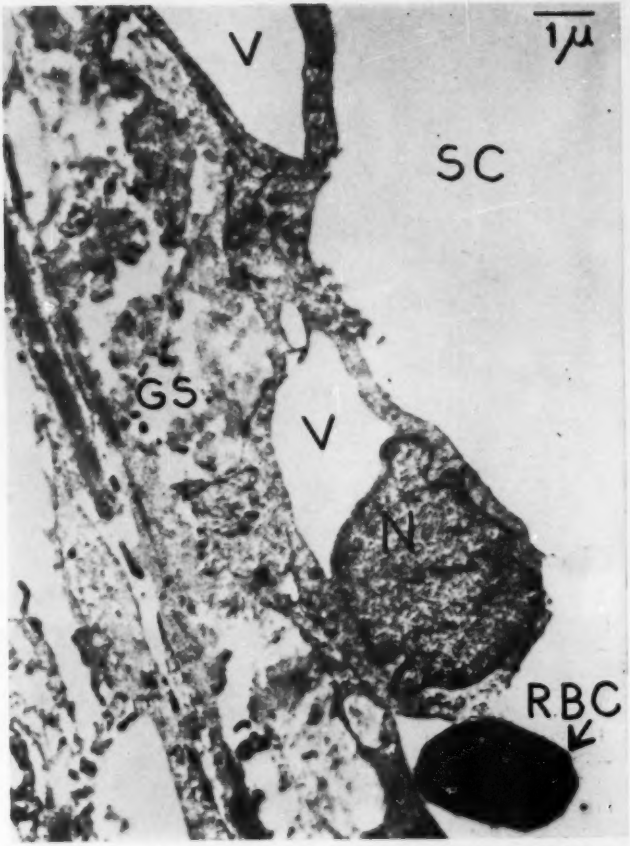
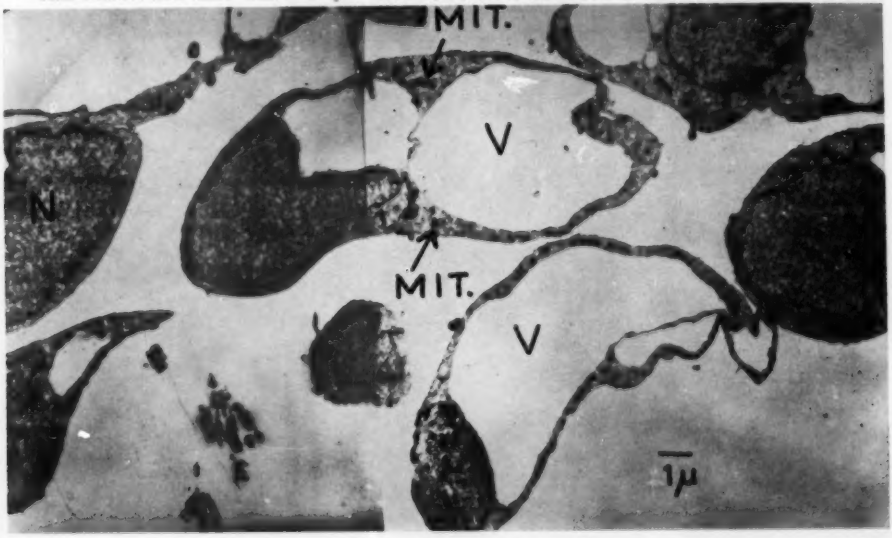


Fig. 17.—(523-24A) Electron micrograph of inner wall of Schlemm's canal showing "vacuoles" in endothelium. *SC*, Schlemm's canal; *V*, "vacuoles"; *GS*, ground substance; *N*, nucleus of endothelial cell; *RBC*, portion of red blood cell in canal; ×11,000; reduced 12% of the original.

Fig. 18.—(523-46 A and B) Electron micrograph montage of several "vacuolated" endothelial cells on inner wall of Schlemm's canal. *N*, nucleus; *V*, vacuoles; *MIT*, mitochondria. These cells connect with inner wall in deeper sections; ×6,000; reduced 16% of the original.



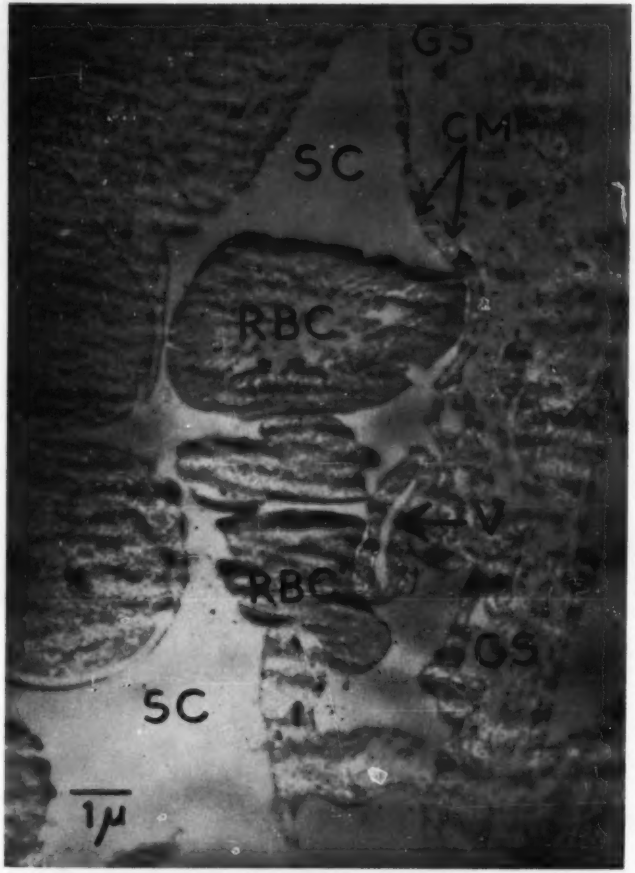


Fig. 19.—(648-4D) Electron micrograph of inner wall of Schlemm's canal. Phosphotungstic acid treatment reveals fibrillar components of ground substance beneath endothelium. *SC*, Schlemm's canal; *N*, nucleus; *CYT*, cytoplasm of endothelium; *TS*, trabecular space; × 18,000; reduced 14% of the original.

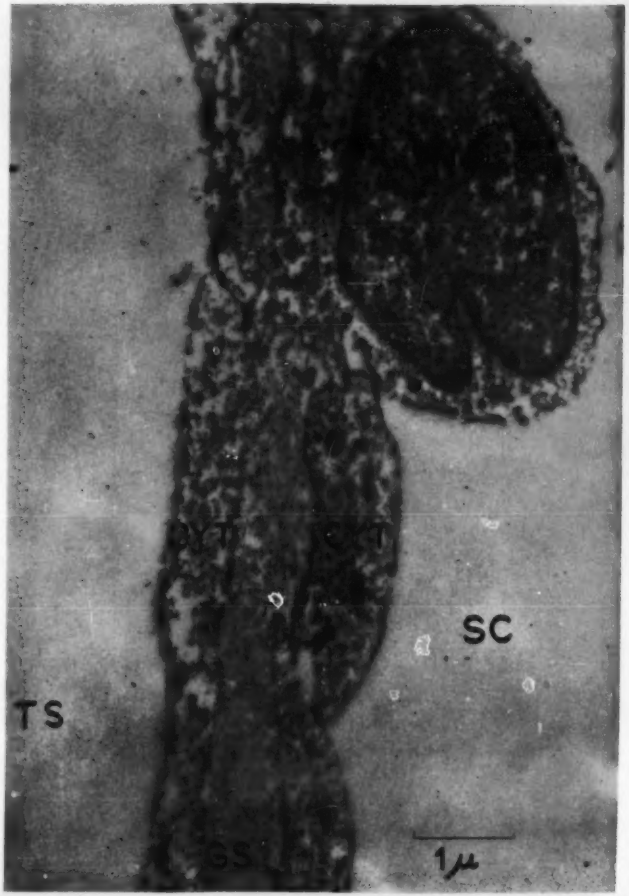


Fig. 20. — (754-14) Electron micrograph showing red blood cells in Schlemm's canal and one incarcerated in passageway into trabeculae. *SC*, Schlemm's canal; *CM*, cell membranes; *GS*, ground substance; *RBC*, red blood cell in Schlemm's canal; *RBC'*, incarcerated red blood cell; × 11,000; reduced 14% of the original.

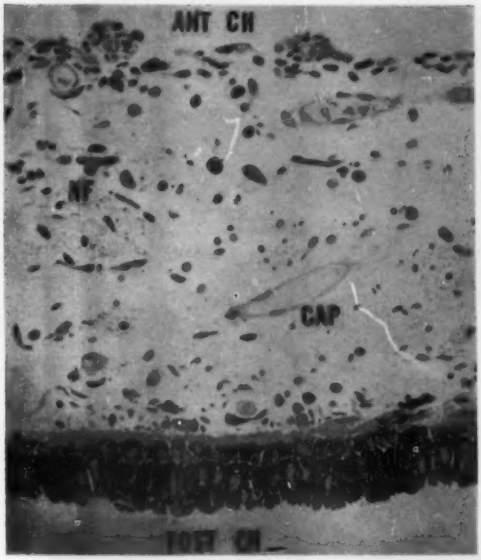


Fig. 1.—Light micrograph of a radial section (1.5μ thick) of rhesus monkey iris near the sphincter muscle. The anterior surface consists of loosely distributed pigmented and nonpigmented cells, similar to the cells found throughout the stroma. Blood vessels in cross and oblique sections are found throughout the iris stroma. A group of nerve fibers (*NF*) mixed with stromal cells is seen near the left center of the micrograph. Adjacent to the dilator smooth muscle layer are the anterior and posterior layers of the pigment epithelium. Large pigment granules may be seen clearly in the cytoplasm of these cells. Osmium tetroxide fixation, methacrylate embedding, Giemsa staining; $\times$ 305.

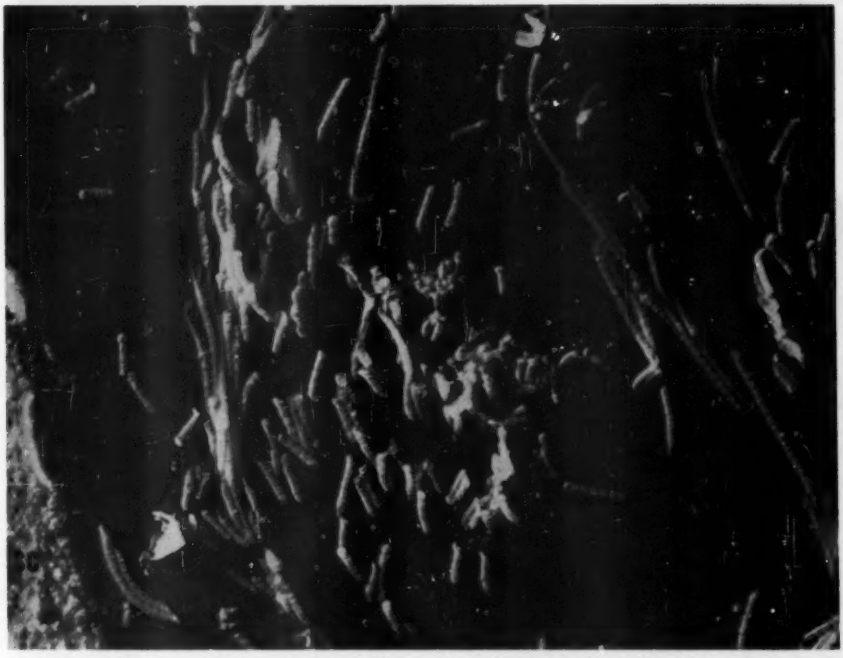


Fig. 2.—Electron micrograph showing stromal collagen fibrils (*CF*) with their characteristic 500-600 A. average axial periodicity. On the left is a thick portion of a stromal cell (*SC*). Rhesus monkey iris; $\times$ 26,000.

The figures on pp. 1077 to 1087 are for the article on pp. 974 to 976 by Tousimis and Fine.

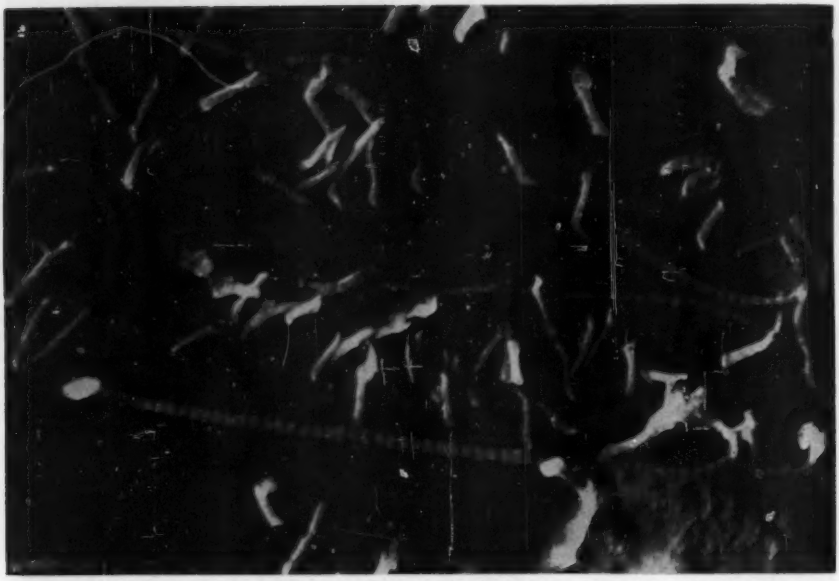
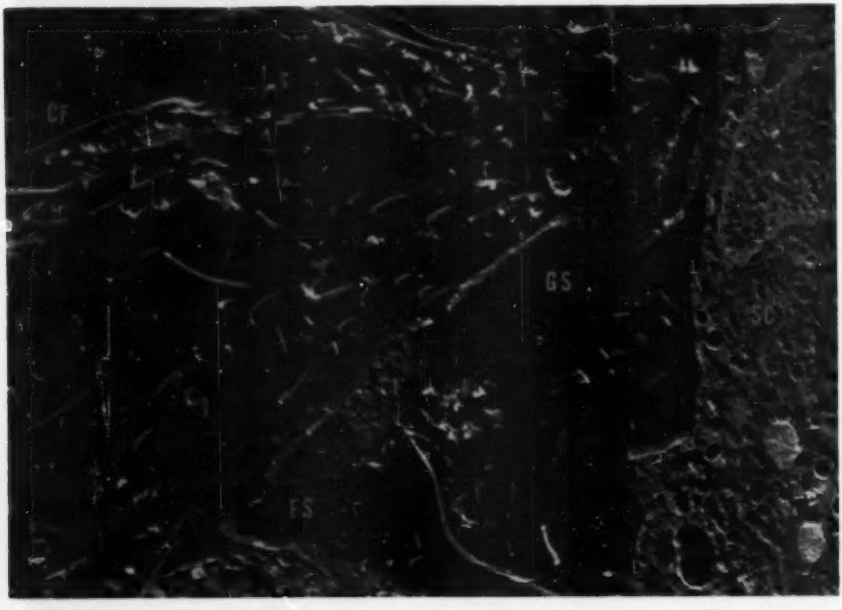


Fig. 3.—Collagen fibrils (*CF*) in the stroma of adult human iris. These fibrils have axial periodicities similar to those found in the monkey iris stroma; × 31,600.

Fig. 4.—Portion of iris stroma (2-month-old child) near the dilator muscle. On the right is a part of a stromal cell (*SC*). Collagen fibrils (*CF*) are present in the stroma. Distributed among them are fine interfibrillar filaments (*FS*) and granules (*GS*); × 13,000.



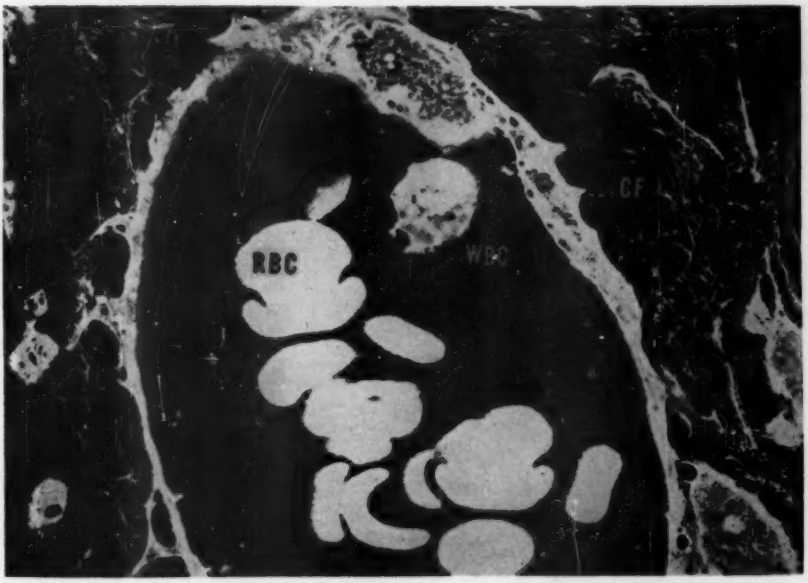
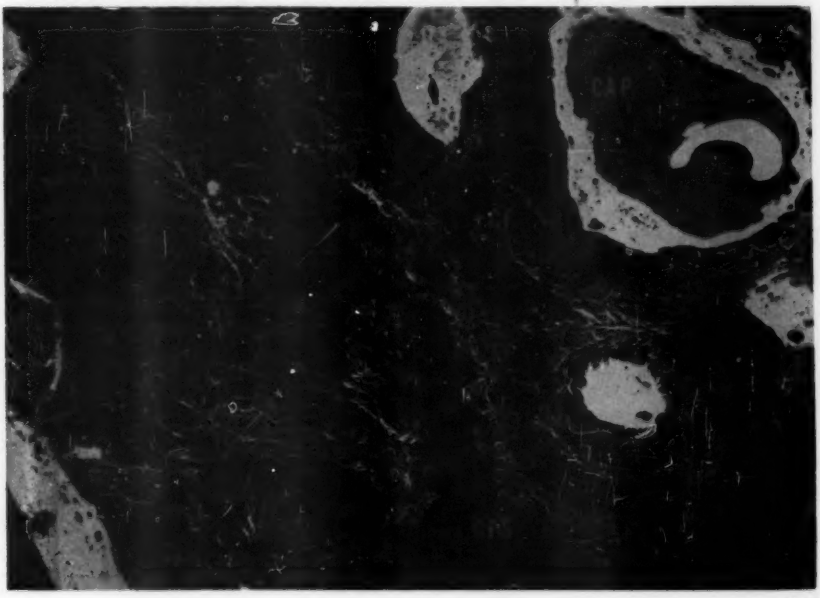


Fig. 5.—A typical large stromal blood vessel in cross section, found in the iris of the human adult. A large number of red blood cells (*RBC*) and one white blood cell (*WBC*) can be seen suspended in the center of the lumen. The vessel wall consists of the cell body and protoplasmic extensions of endothelial cells one layer thick. Surrounding the vessel, which has the characteristics of a large capillary, are the loosely arranged collagen fibrils (*CF*); × 2,830.

Fig. 6.—Elongated "spaces" or "canals" are frequently found between adjacent aggregates of stromal components. One of these traverses the field from the upper left to the lower right corner. Both the upper and lower aggregates in this micrograph consist of a blood vessel (*CAP*), stromal cells, and collagen fibrils (*CF*). Drying patterns of some of the interfibrillar substance (*GFS*) can be seen throughout the electron micrograph and the lengthy "space." Human adult iris; × 2,830.



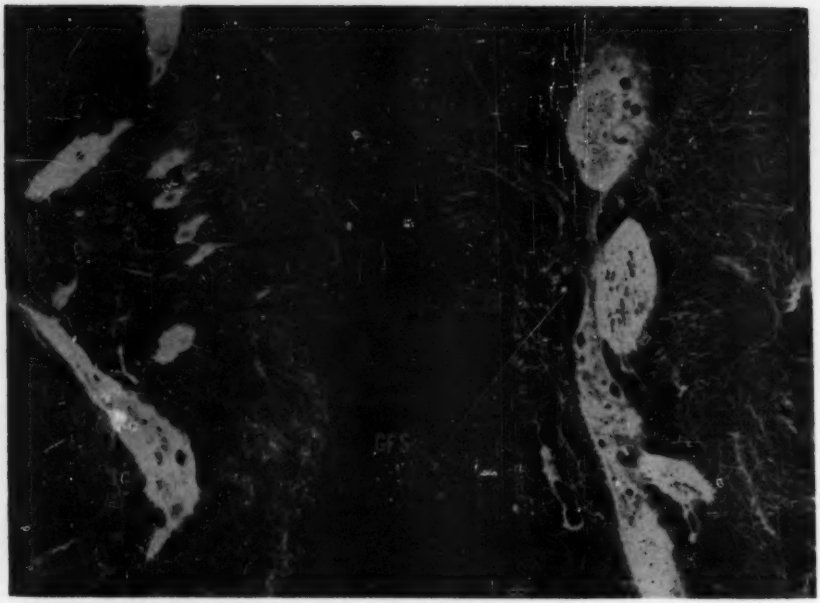
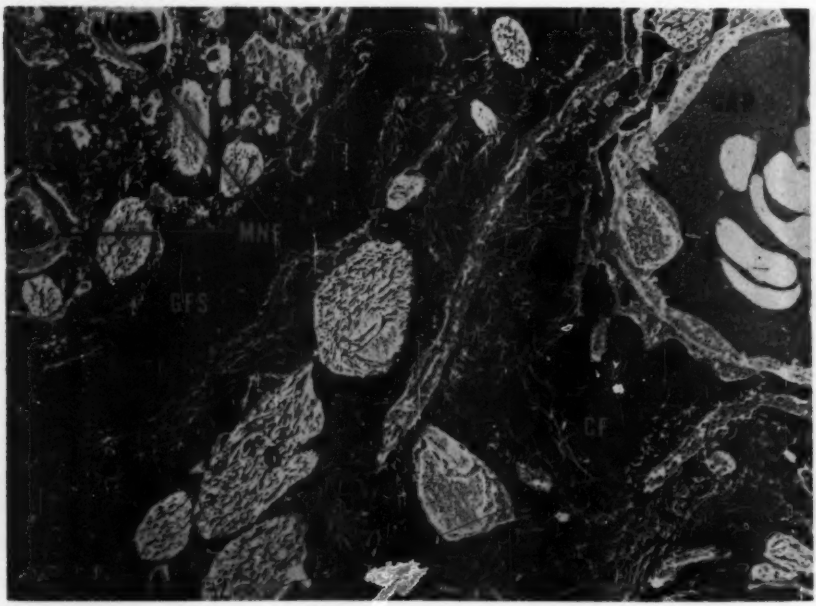
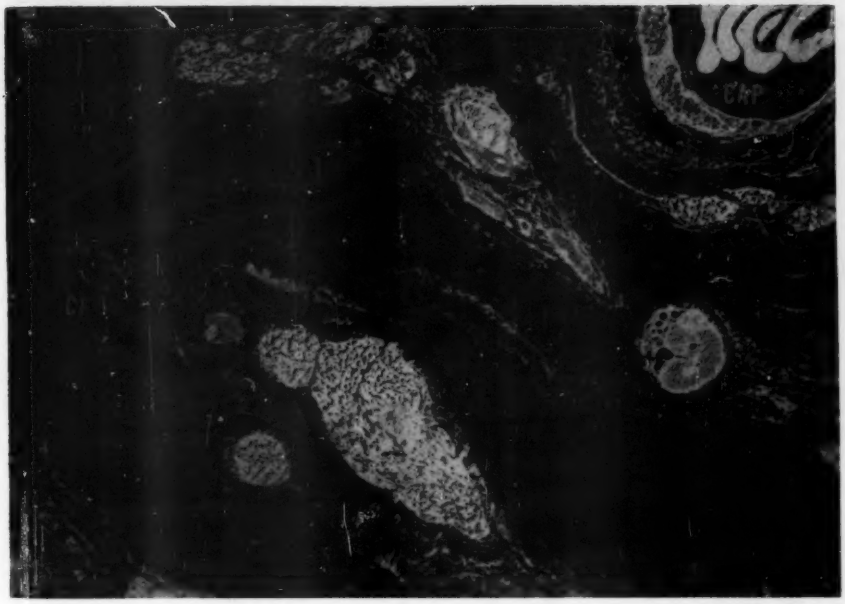


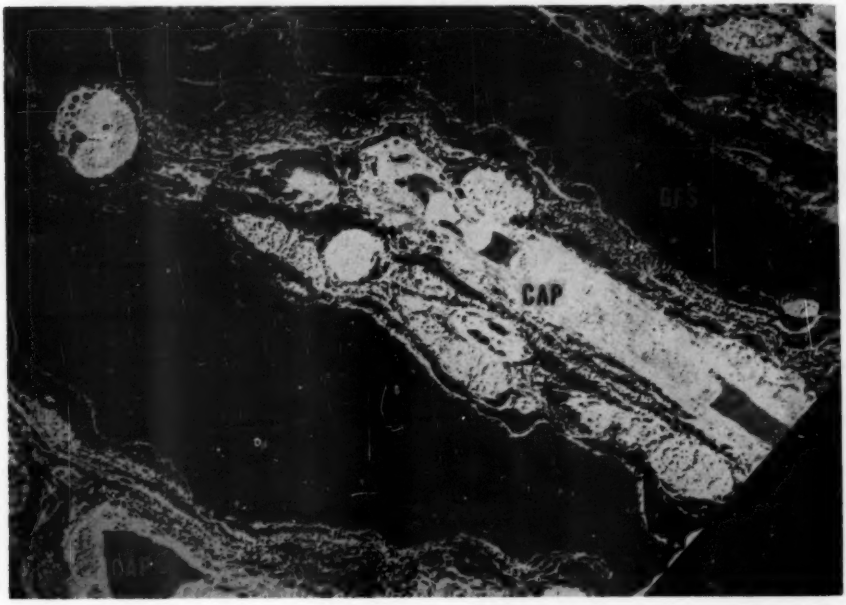
Fig. 7.—A micrograph of adult human iris showing the "space" or "canal" present between two aggregates of stromal tissue. Within these aggregates are sectioned stromal cells. The drying patterns of interfibrillar substance (*GFS*) can be observed within the "space" and throughout the adjacent stromal tissue aggregates; × 2,830.

Fig. 8.—Two adjacent stromal aggregates are shown in this micrograph of rhesus monkey iris. The lower aggregate consists of a large iris capillary (*CAP*) and its surrounding complement of pigmented and nonpigmented stromal cells and loose collagen fibrils (*CF*). The upper stromal aggregate consists of a bundle of myelinated nerve fibers (*MNF*), stromal cells, and collagen fibrils. Interfibrillar material (*GFS*) is seen in the larger "spaces," particularly in the long "space" between stromal aggregates. Rh monkey, middle one-third of stroma towards sphincter muscle; × 2,830.





Figs. 9 and 10.—Micrographs of rhesus monkey iris sectioned tangential to pupil about the middle one-third of the iris near the dilator muscle. Figure 10 is a continuation of Figure 9. Several aggregates are separated by long "spaces," in one of which interfibrillar material (*GFS*) can be seen clearly. A capillary (*CAP*) in cross section is present in one of the aggregates or stromal bundles in Figure 9. Two such capillaries are seen in Figure 10, one of which has been sectioned somewhat longitudinally (*CAP*). Pigmented and nonpigmented stromal cells and collagen fibrils (*CF*) are in the stromal bundles; × 2,830.



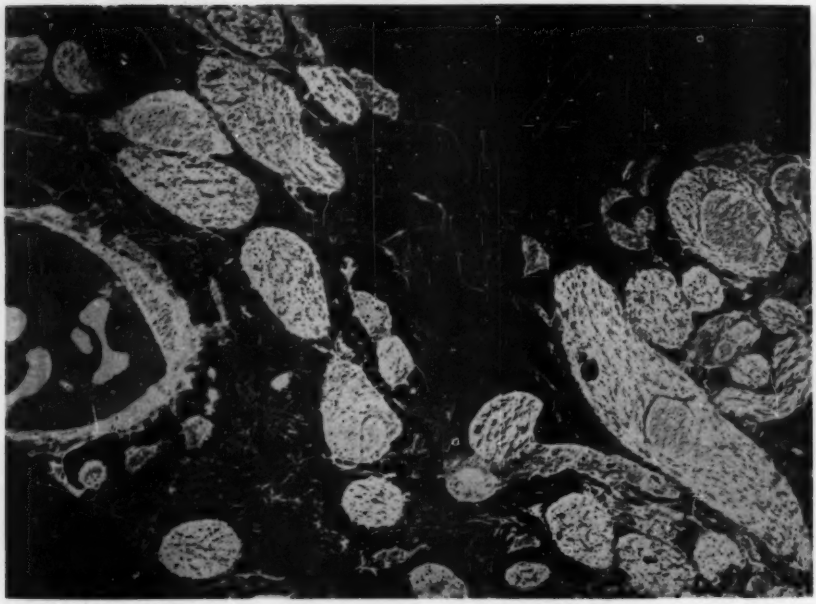
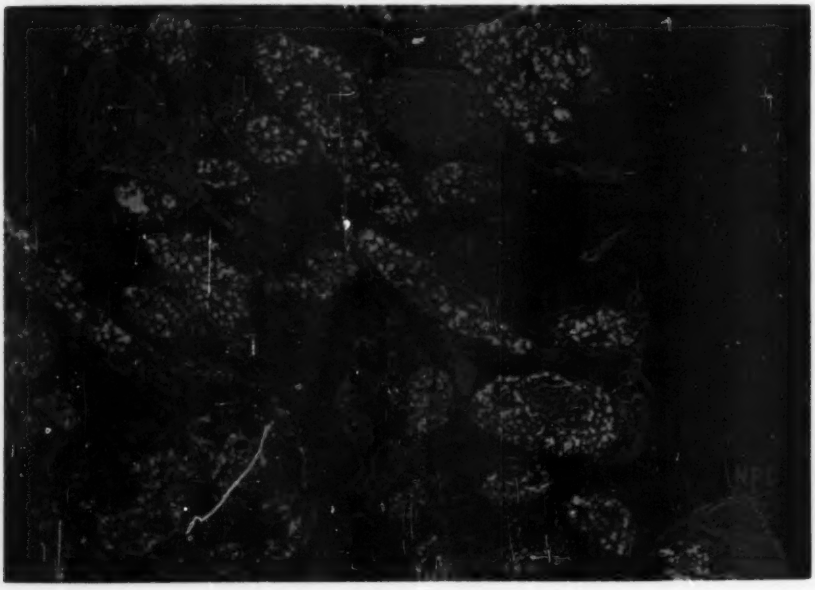


Fig. 11.—Anterior surface of the rhesus monkey iris consists of "loosely" arranged pigmented and nonpigmented stromal cells in a matrix of collagen fibrils. Blood vessels (capillaries) are found near the surface (*CAP*). Numerous apertures—portions of the iris surface without cells—have been observed in both monkey and human irides. A large aperture is seen in this electron micrograph leading from the anterior chamber into the iris stroma (*arrows*). Collagen fibrils are loosely distributed within it. Similar fibrils are seen—without specific orientation—between the stromal cells; × 2,830.

Fig. 12.—Anterior surface of human iris (2-month-old child) is composed of both pigmented (*PC*) and nonpigmented (*NPC*) cells with intercellular collagen fibrils (*CF*). These stromal cells, which form the anterior surface of the iris, are loosely arranged without any specific orientation. The apertures (*arrows*), originating on the surface, lead into interstitial spaces within the interior of the structure; × 2,830.



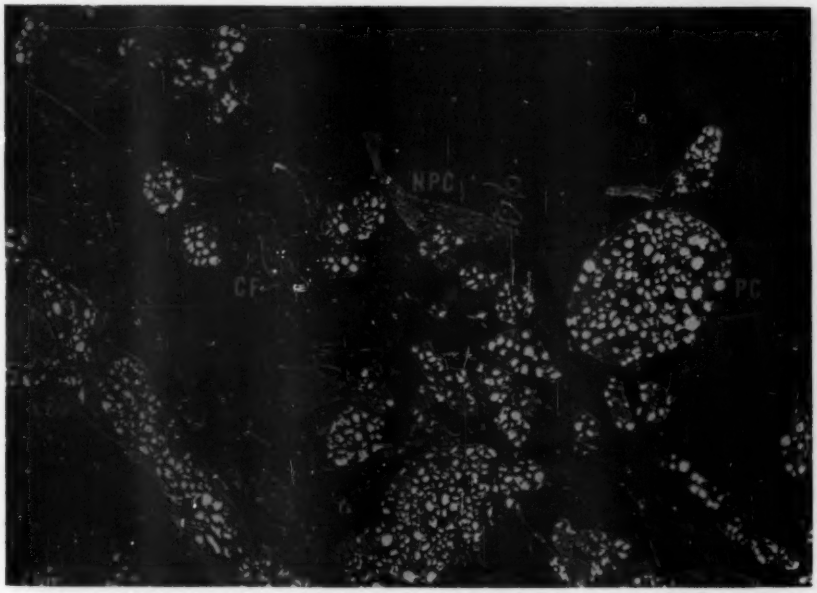
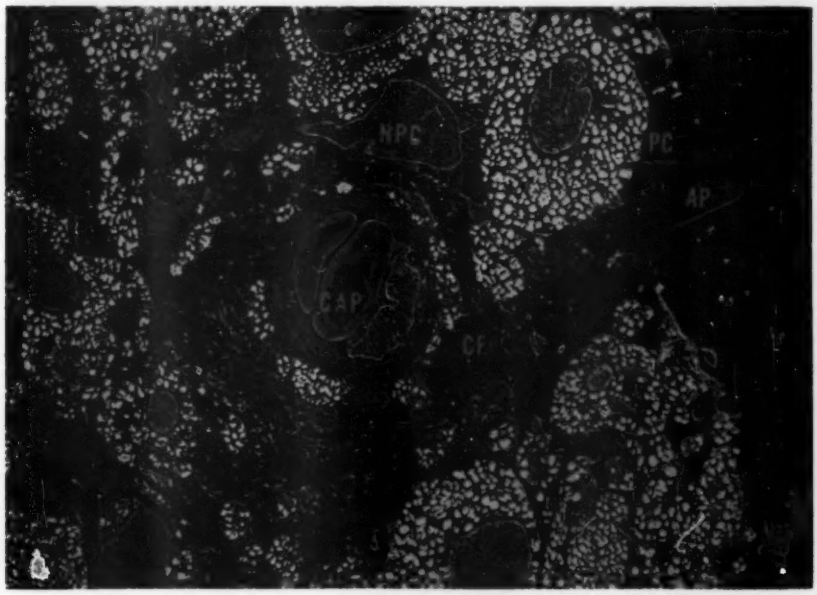


Fig. 13.—Anterior surface of adult human iris near the pupillary zone is composed of pigmented (*PC*) and nonpigmented cells (*NPC*), with relatively few collagen fibrils (*CF*) in between. Stromal cells with long cytoplasmic processes are present in the surface layer, as shown in this electron micrograph. These processes are cut both longitudinally and tangentially in this radial section of iris. Openings on the surface, resulting from such a loose arrangement of these cells, continue into the stroma proper; × 2,830.

Fig. 14.—Anterior surface of the adult human iris near the sphincter muscle in some areas appears to be composed of more closely arranged stromal cells (*PC, NPC*). These cells do not have any true interdigitation. An aperture (*AP*) leads into the interior of the iris. The blood vessel (*CAP*) is very close to the surface and literally in the way of the intercellular space formed by the loose arrangement of the stromal cells. Collagen fibrils (*CF*) are seen in between the cells; × 2,830.



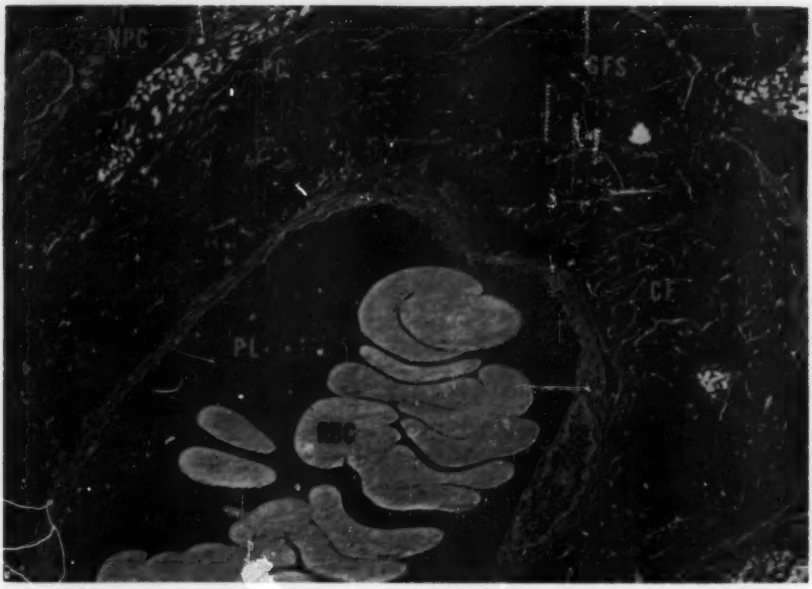
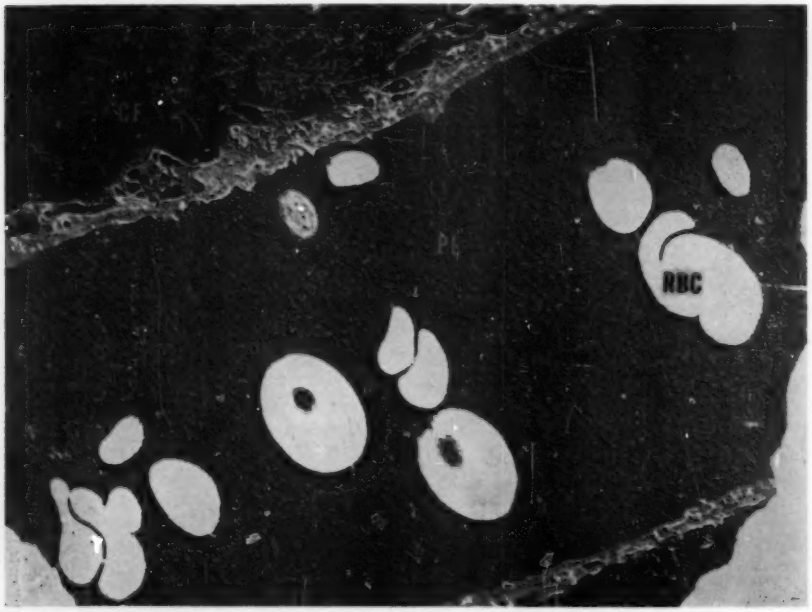


Fig. 15.—Electron micrograph showing a stromal blood vessel of a child's iris, collagen fibrils (*CF*), an abundance of interfibrillar granular and filamentous substance (*GFS*), and pigmented (*PC*) and nonpigmented cells (*NPC*). Red blood cells (*RBC*), surrounded by granular-appearing plasma (*PL*), are packed in the center of the vessel. Collagen fibrils surrounding the vessel do not have any specific orientation with respect to the neighboring cells or the vessel; × 2,830.

Fig. 16.—Longitudinal section of a blood vessel in the rhesus monkey iris. Its thin walls, characteristic of capillaries, are similar to those of the human iris. Red blood cells (*RBC*) are seen in the lumen in the center of the vessel suspended in the granular-appearing plasma (*PL*). Surrounding the capillary, in random orientation, are collagen fibrils (*CF*) and interfibrillar substances; × 2,830.



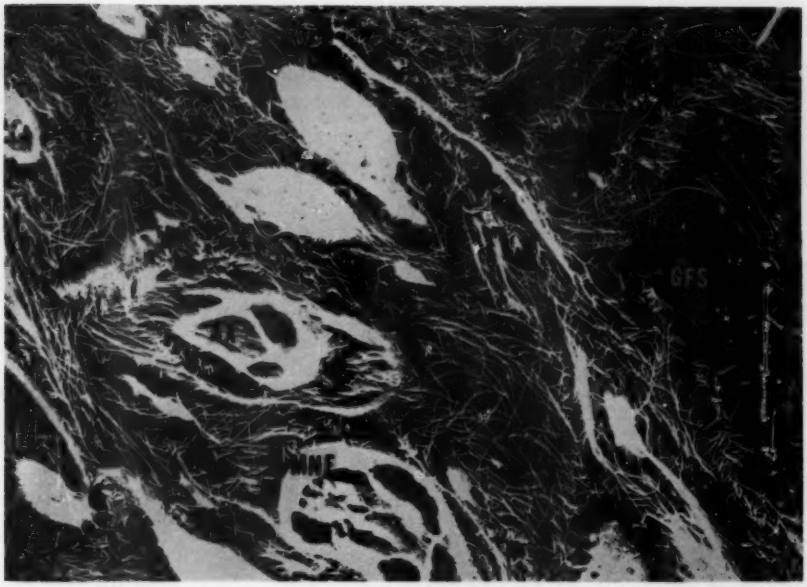
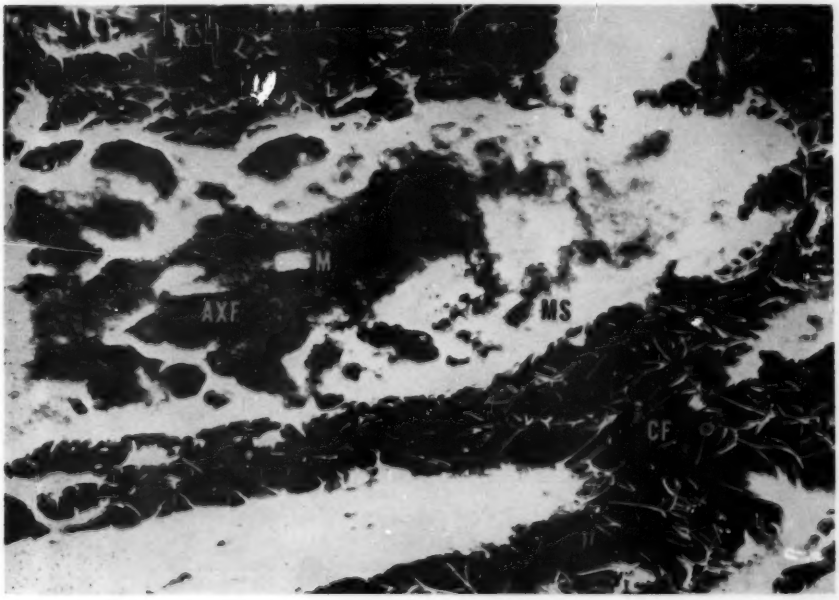


Fig. 17.—Electron micrograph of a stromal bundle in the adult human iris containing myelinated nerve fibers (*MNF*) cut in cross section, together with aggregates of stromal cells and collagen fibrils. Interfibrillar material (*GFS*) can be seen in the "spaces" of the upper right portion of the micrograph; × 2,830.

Fig. 18.—A myelinated nerve fiber cut longitudinally in the stroma of the rhesus monkey iris. The typical laminations of the myelin sheath (*MS*) are not demonstrable in this "thick" section. The axoplasm, however, is seen to consist of many small filaments. A few narrow mitochondria (*M*) in linear arrangement are present in the axoplasm of the nerve fiber. Around the nerve are the collagen fibrils (*CF*), with an axial periodicity of 500-600 A., and portions of stromal cells; × 7,600.



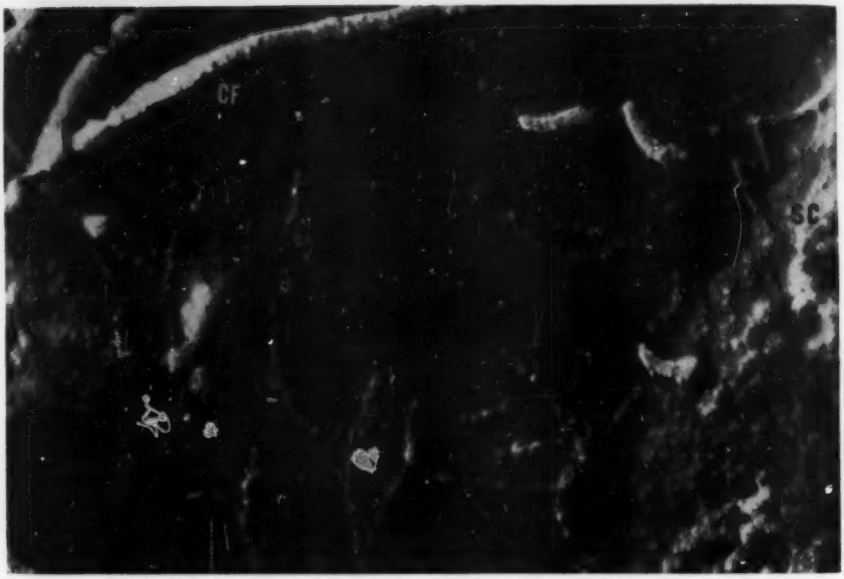
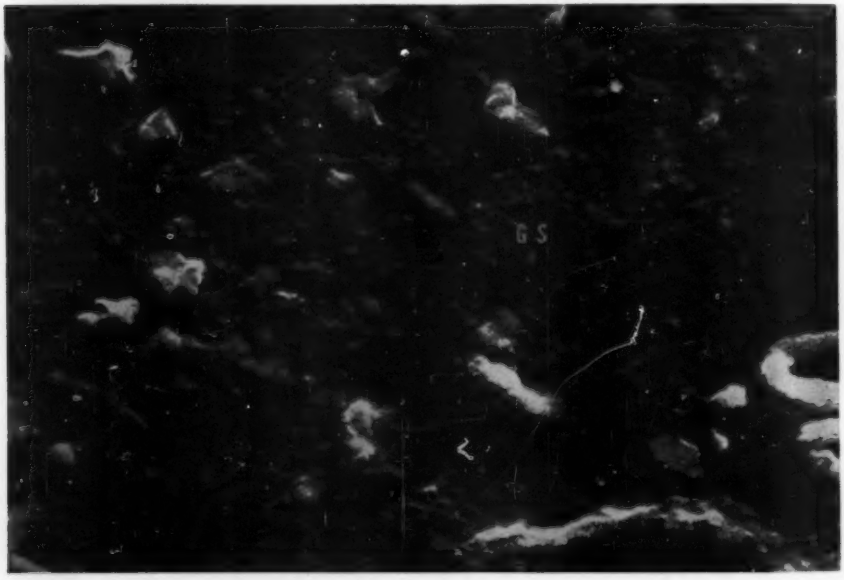
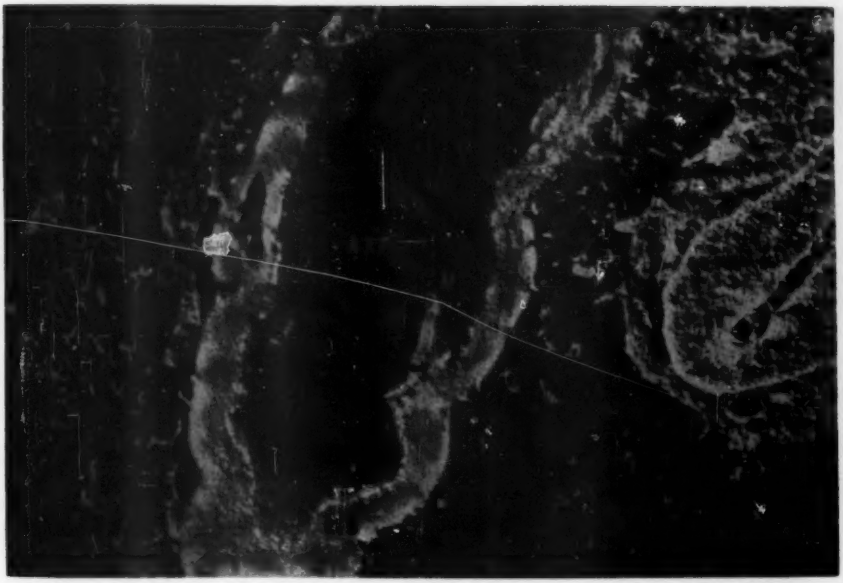


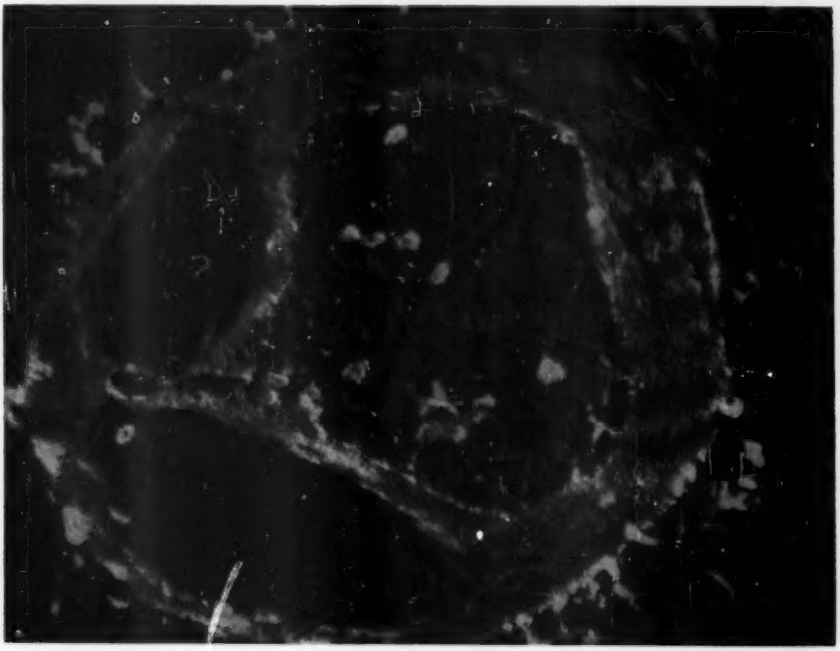
Fig. 19.—A higher magnification micrograph of a portion of Figure 4, showing some of the intercellular contents of the iris stroma of a 2-month-old child. Collagen fibrils (*CF*) are present on the upper left and a portion of a stromal cell (*SC*) on the right. In the center of the field, fine beaded filaments are seen; × 60,000.

Fig. 20.—A higher magnification micrograph of a portion of Figure 4, showing the intercellular granular substance (*GS*) found between collagen fibrils in the stromal "spaces"; × 60,000.





Figs. 21 and 22.—Longitudinal and cross section, respectively, of myelinated nerve fibers from the rhesus monkey iris. Their axoplasm contains long, thin, mitochondria (*M*) oriented in the direction of the long axis of the nerve. The beaded filaments (*AXF*) also show similar orientation. The laminated character of the myelin sheath (*MS*) can be observed. Surrounding the nerve fiber are collagen fibrils and/or stromal cells; Figure 21 × 13,000; Figure 22 × 23,000.



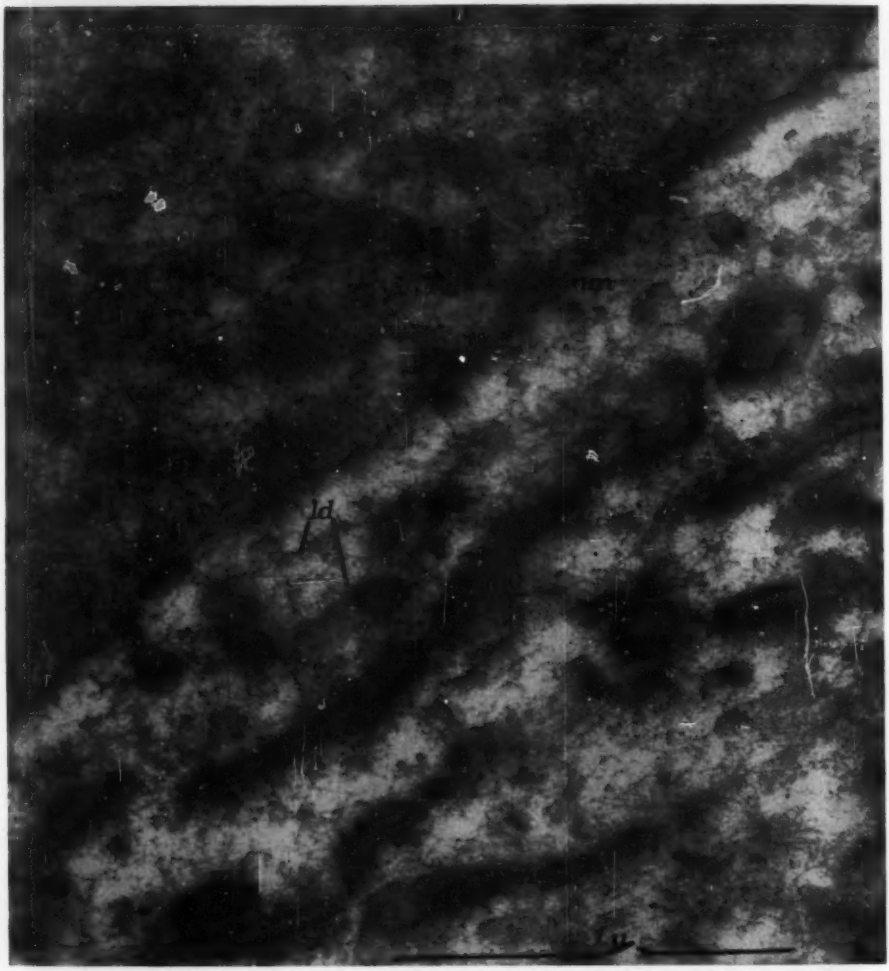
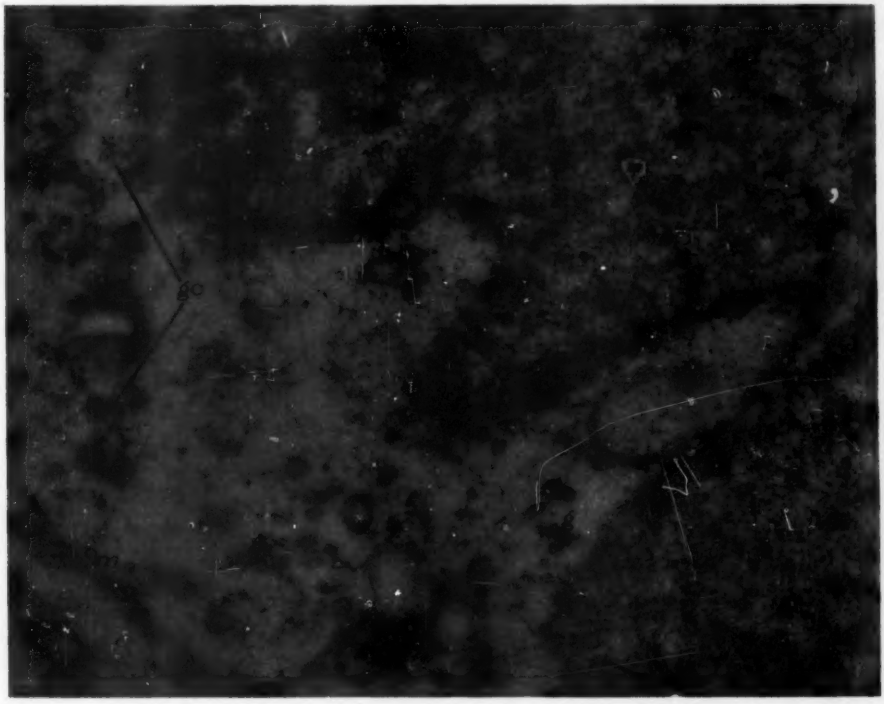


Fig. 1.—Lens epithelium, central zone, 24 hours after irradiation: The cytoplasm appears unchanged and contains formed constituents, such as rough surfaced endoplasmic reticulum (*cer*), mitochondria (*m*), and elements of low density (*ld*). In the left upper half of the picture, the nucleus (*n*) displays a nucleolus (*nn*) which immediately adjoins the nuclear membrane (*nm*). Approximately × 62,000.

The figures on pp. 1088 to 1095 are for the article on pp. 977 to 984.

Fig. 2.—Lens epithelium, equatorial zone, 10 days after irradiation: A definitely binucleated cell in the left half of the picture shows two nuclear profiles (n_1, n_2) with indistinct boundaries. A crenated nucleus (n) appears in tangential section on the right and exhibits several orifices (arrow) along one circumference. In the center of the picture, the plasma of a cell process (between cm_2 and cm_3) contains a profusion of low density structures. Approximately × 10,000.

Fig. 3.—Lens epithelium, equatorial zone, 10 days after irradiation: The right half of the picture is occupied by a nucleus (n), with several deep indentations along its surface and with the nucleolus (nu) located in one of these nuclear projections. Profiles of the Golgi complex (gc), cell membranes (cm), dense granules (g), and nuclear membrane (nm) are indicated. Approximately × 37,600.



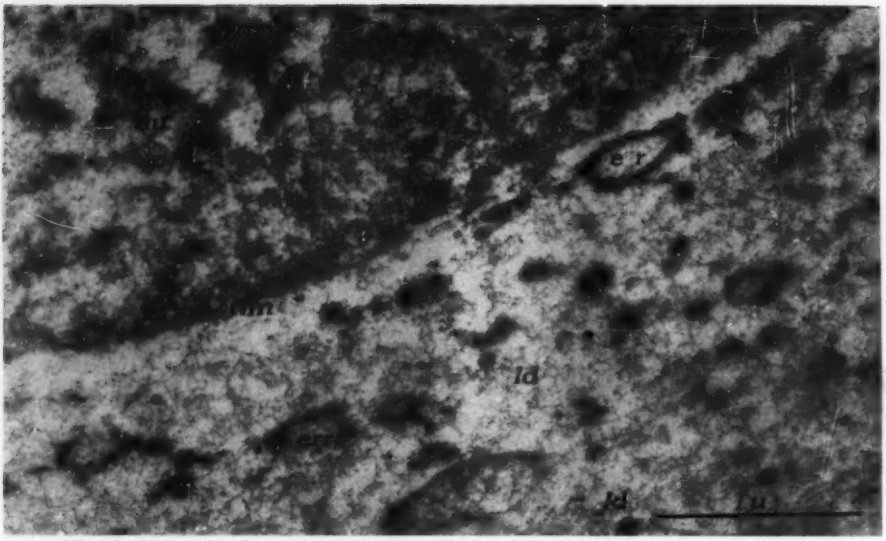
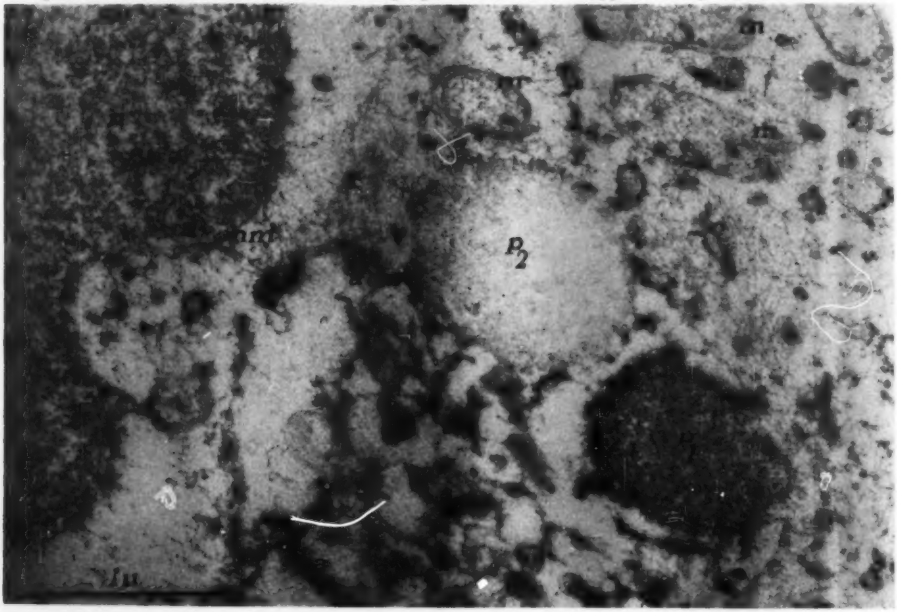


Fig. 4.—Lens epithelium, equatorial zone, 14 days after irradiation: The cytoplasm contains mainly vesicular profiles of the rough surfaced endoplasmic reticulum (*err*) and elements of low density (*ld*). A nucleolus (*nu*) is seen at the periphery of the nucleus (*n*) with its granular component irregularly dispersed along the nuclear boundary (*nm*). Approximately × 32,250.

Fig. 5.—Lens epithelium, equatorial area, 10 days after irradiation: A nucleus (*n*), with an intact nuclear membrane (*nm*), is adjacent to an agglomerate consisting of rounded particles showing various densities (P_1, P_2) and of irregular, membranous, and opaque profiles (*a*). The formation at P_1 is similar to structures interpreted as disintegrating nuclei in deeper lens fibers. Mitochondria (*m*) show finely granulated matrices; their membranous portions are less dense and more widely spaced than usual. Approximately × 32,250.



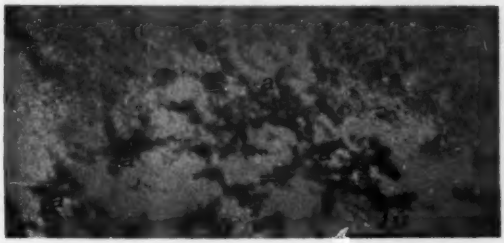
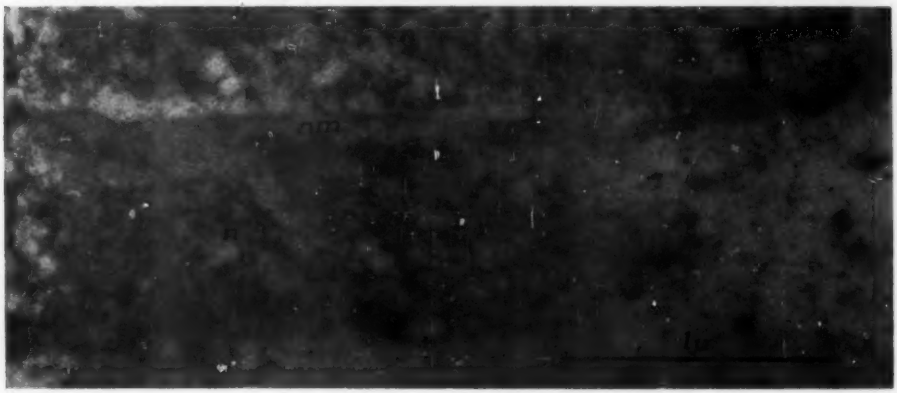


Fig. 6.—Lens epithelium, peripheral zone, 24 hours after irradiation: In this instance, the nuclear membrane is absent between the nucleoplasm (*n*) and this paranuclear agglomerate similar to that seen in Figure 5. Dense components of the agglomerate are indicated at (*a*). Approximately × 19,000.

Fig. 7.—Lens epithelium in a flat mount, Feulgen-stained, 10 days after irradiation: Rounded Feulgen-positive clumps (*a*) are seen in the cytoplasm adjacent to nuclei. They appear surrounded, in part, by a halo-like profile. Light micrograph, Reichert-Zetopan, approximately × 1,012.

Fig. 8.—Lens epithelium, equatorial zone, 10 days after irradiation: A small accumulation of nucleoplasm (*n'*) adjoins the surface of a nucleus (*n*). Where the small nucleoplasmic aggregate is attached, the space between the dense lamellae of the nuclear membrane (*nm*) appears distended. Approximately × 43,000.



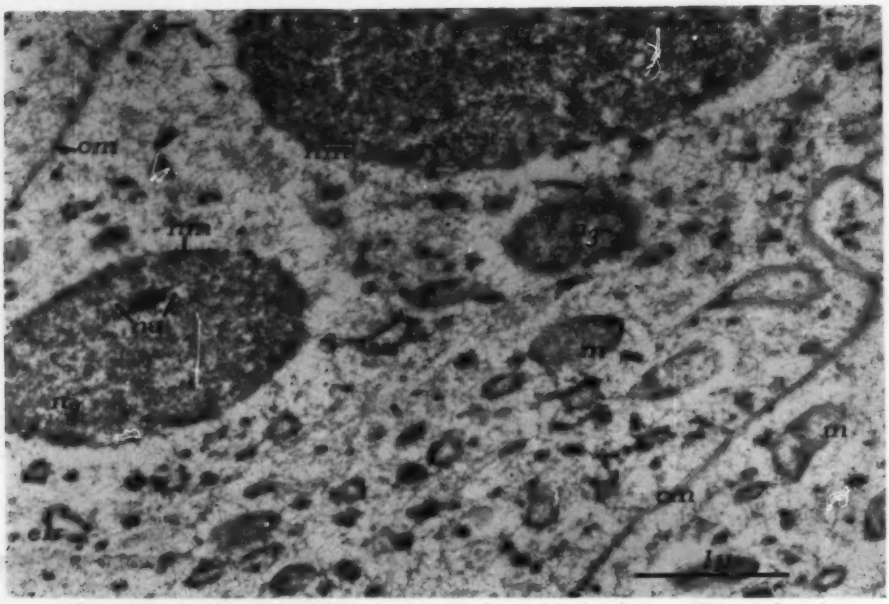


Fig. 9.—Lens epithelium, peripheral zone, 14 days after irradiation: Three independent nuclear configurations are present in this one cell. One (n_1) is larger than the others (n_2, n_3) and might be the original nucleus. The nuclear profile at n_3 contains an accumulation of dense granular material, comparable to a nucleolus (*nu*). Rough-surfaced endoplasmic reticulum (*err*), mitochondria (*m*), cell membranes (*cm*), and nuclear membranes (*nm*) are designated. Approximately × 23,900.

Fig. 10.—Lens epithelium, flat mount, Feulgen-stained, 14 days after irradiation: Several Feulgen-positive configurations (accessory nuclei) (*n'*) stain with an intensity similar to that of the main nucleus (*n*) in the cell. Light micrograph, Reichert-Zetopan, approximately × 1,012.

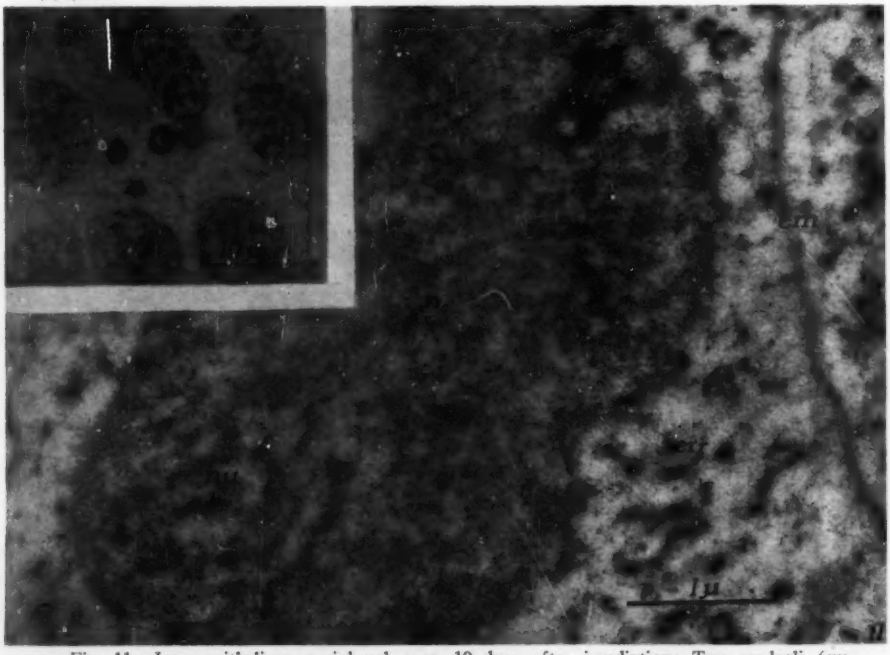


Fig. 11.—Lens epithelium, peripheral zone, 10 days after irradiation: Two nucleoli (*nu*, *nu'*) are located at opposite poles of the same nucleus (*n*). A nuclear membrane is not distinguishable. Rough-surfaced endoplasmic reticulum (*err*) and cell membranes (*cm*) are evident. Approximately × 23,900.

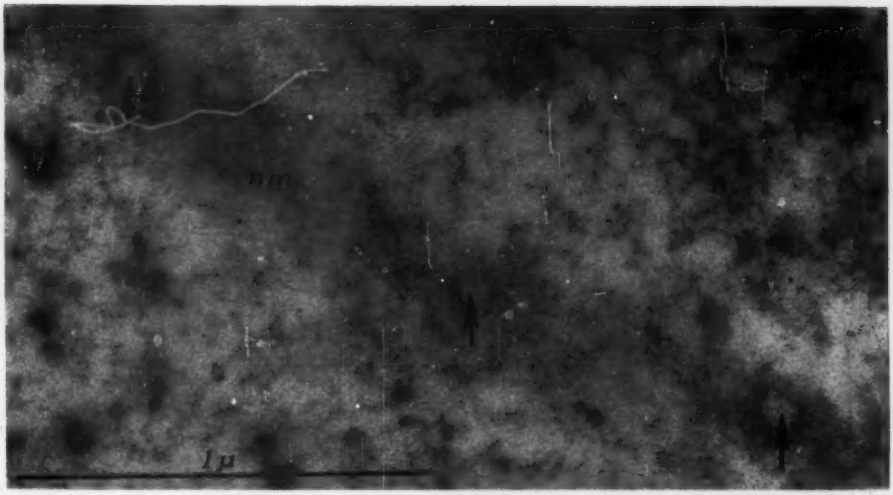


Fig. 12.—Lens epithelium, central zone, 14 days after irradiation: The nucleoplasm is condensed into irregular thick clumps (*n*), leaving a light zone along the inner surface of the nuclear boundary. The nuclear membrane (*nm*) appears band-like in this section and has circular stomata (arrows) disposed along its circumference. Approximately × 64,500.

Fig. 13.—Lens epithelium, central part, 14 days after irradiation: Here, rough-surfaced endoplasmic reticulum (*err*) shows an increased density of its lumen (*l*) and numerous attached, dense granules (*g*). Thus, there is a marked difference in appearance compared with its configuration in Figure 1. Approximately × 46,650.

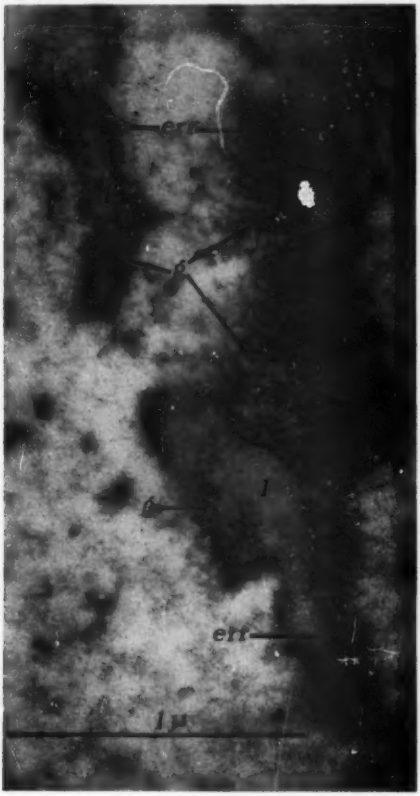


Fig. 14.—Lens epithelium, peripheral zone, 10 days after irradiation: The increase in the distance between cell membranes is represented in this figure (arrows). Endoplasmic reticulum (*err*) and interdigitating cell processes (*int*) are indicated. Approximately × 46,650.

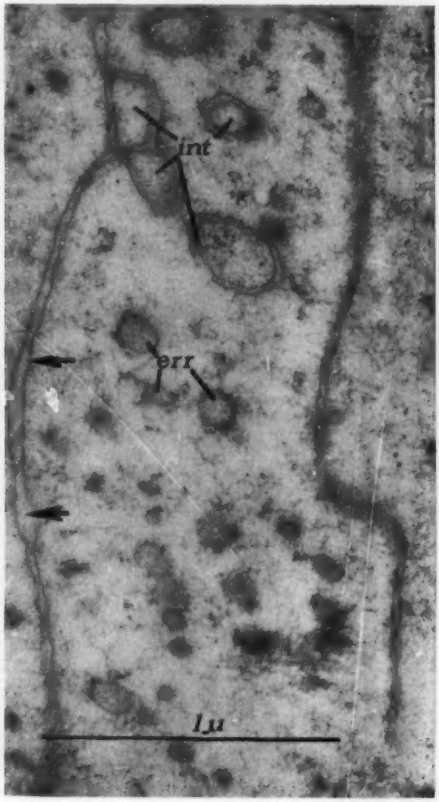
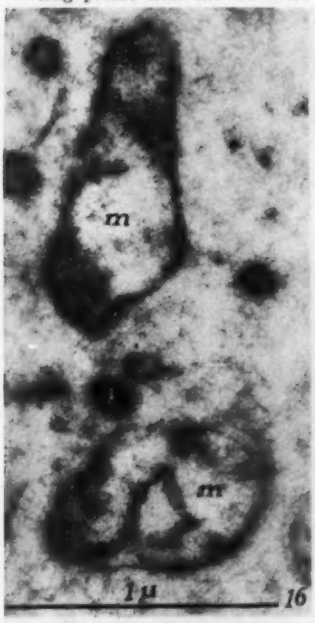
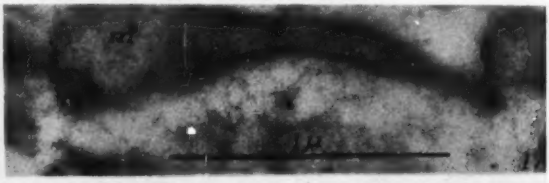
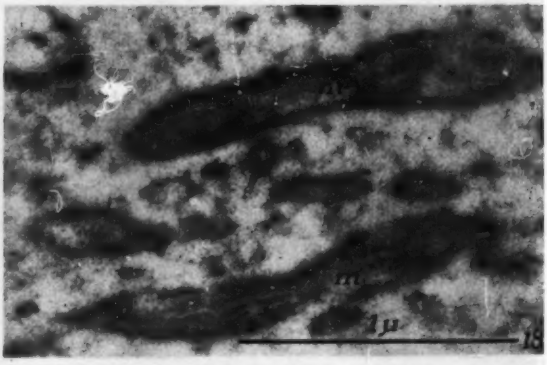


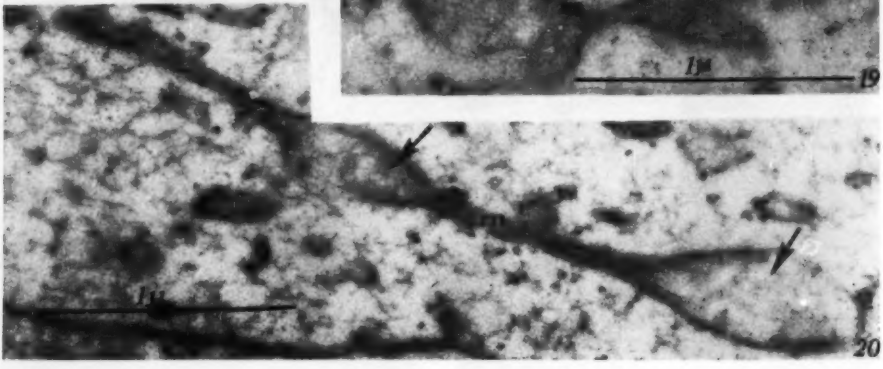
Fig. 15.—Lens epithelium, peripheral zone, four days after irradiation: An elongated body (*m*), composed of several lamellae (arrow) of a dense, membranous nature, is found in the cytoplasm at this interval. Approximately × 64,500.

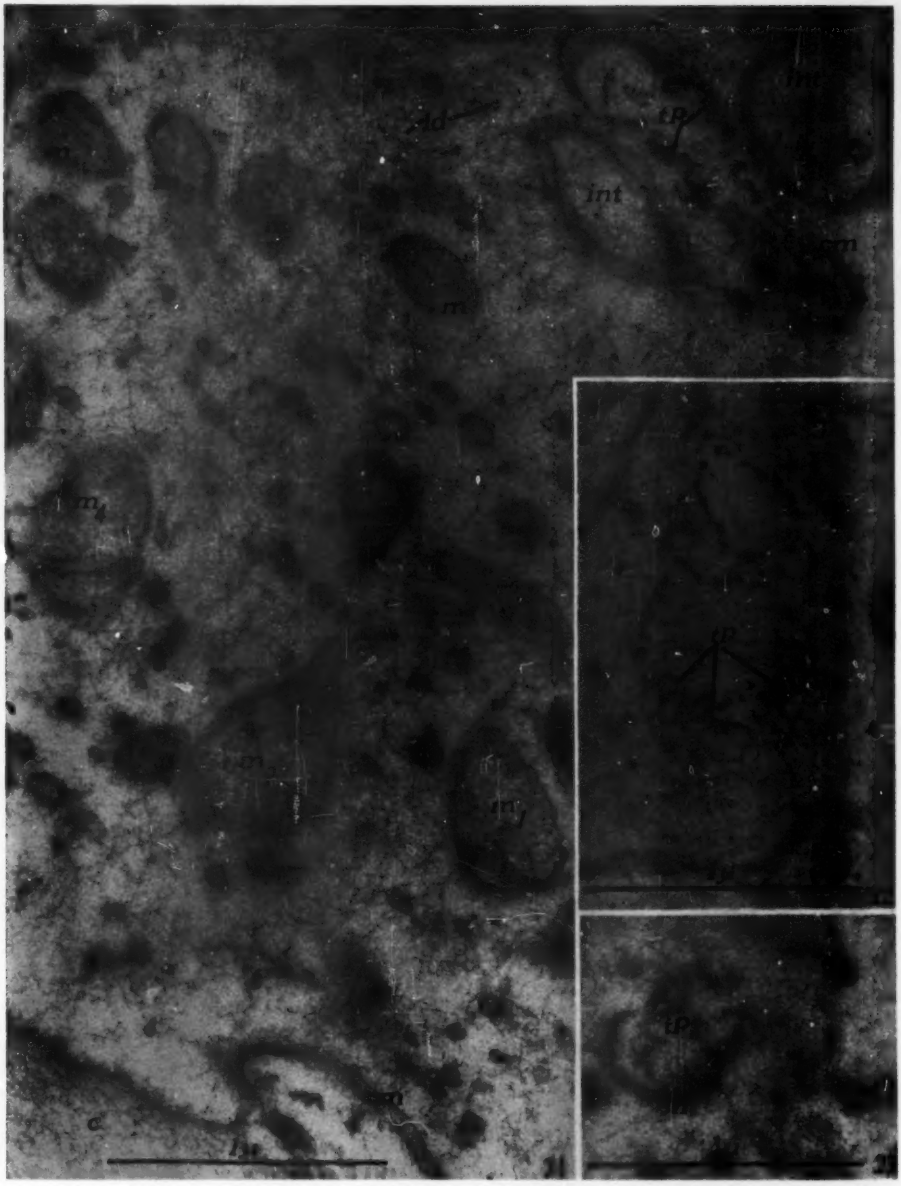
Figs. 16-20.—Lens epithelium, peripheral zone, 10 days after irradiation: Mitochondria (*m*) assume many different forms. In Figure 16, they seem distended and their cristae are displaced towards the periphery. A club-shaped structure is seen in Figure 17, while in Figure 18 mitochondria have an elongated shape, with long cristae similar to mitochondria seen in normal fibers. Figure 19 shows a mitochondrion of bizarre outline, and Figure 20 a long profile with two distended portions (arrow). Approximately × 43,000.











Figs. 21-23.—Lens epithelium, peripheral zone, 10 days after irradiation: Part of a cell, near the lens capsule (*c*). In the upper right corner, dense, tortuous, membranous profiles (*tp*) in an elliptical arrangement can be seen between two interdigitating cell processes (*int*). Similar structures are depicted in Figures 22 and 23. These short, tortuous profiles are closely associated with longer, double membranous structures similar to cell membranes (*cm*). Mitochondria (*m*) exhibit various changes, such as increased granularity of the matrix (m_1, m_2), distended lumen of the cristae (m_1, m_2, m_4), and a general decrease in opacity (m_4). Clusters of dense granules and elements of low density in the cytoplasmic matrix are designated. Approximately × 43,000.

Greater Image Magnification in Indirect Ophthalmoscopy

FRANCIS A. L'ESPERANCE Jr., M.D., Boston

Indirect ophthalmoscopy is a method of fundoscopy which has come into increased prominence during the last decade. In this method, a convex lens is held before the patient's eye and an inverted real image of the fundus is produced in the air between the observer and the lens. This image can then be studied in various ways by the observer.

In 1947, Dr. Schepens devised a self-luminous binocular indirect ophthalmoscope [1] which allowed the visual axes, by means of reflecting surfaces within an eyepiece arrangement, to become paraxial with the light source beam. The outstanding feature of this instrument was, and is today, the relatively large fundus field that can be examined stereoscopically and with intense illumination. The magnification of the retinal image with this conventional ophthalmoscope is low, however, and varies as an inverse function of the power of the convex lens used. Any increase in fundus magnification, obtained by reducing the power of this lens, is accompanied by a similar reduction in field. Since it would be advantageous to study fundus lesions binocularly, and in more detail with a greater magnification, I would like to present a modification of the Schepens-AO indirect ophthalmoscope which achieves this goal without any reduction in the fundus field obtained.

This modification applies the magnifying power of a miniature prism binocular to the inverted aerial image produced by the convex lens. The miniature binocular is substituted for the simple eyepiece previously employed.

The most significant feature of the standard instrument, that of stereoscopic vision, is preserved in the modification with a substantial increase (10×) in the stereoscopic effect due to the more widely placed binocular objectives and the inherent magnification of the instrument.

A second feature, which is preserved in this new model, is an arrangement for varying the interpupillary distance by means of movable ocular arms. The interpupillary distance may be quickly and accurately adjusted to any distance from 59 to 74 mm.

A third retained feature of this modification is the complete visualization of the aerial image. That is, any image produced by the convex lens will be seen in its entirety, and the extent of the fundus viewed will depend only upon the convex lens used. With both the conventional and the modified ophthalmoscope, the same aerial image is viewed, but with much greater magnification in the latter case. The fundus field seen with the prism binocular is, therefore, identical with that of the present eyepiece.

The most prominent feature of this modification is the greater magnification of the aerial image. This magnification is approximately eight times that seen heretofore with the conventional instrument. With the 7-power binocular employed in my preliminary model, the actual retinal magnification factor (in an emmetrope) varied from 24 times with the +20 D. hand-held lens to 32+ times with the +14 D. lens. These magnifications are to be com-

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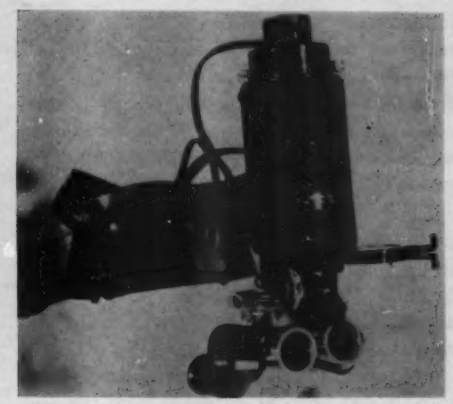

Fig. 1.—An oblique view of the assembled, modified, indirect ophthalmoscope showing the headband, A-O illuminating source, and miniature prism binocular adaptation.

pared with the 3 to 4.5 diameter magnification with similar lenses in the present indirect ophthalmoscopy, and with the usual 15 diameter magnification in direct ophthalmoscopy.

Equipment

Any powerful light source attached to a headband would provide a sufficient base upon which the binocular could be mounted. I have employed the 18 watt light source and headband arrangement of the Schepens-AO indirect ophthalmoscope. In place of the eyepiece unit of this ophthalmoscope, I have substituted a 7-power miniature prism binocular attached by a plastic mount to the light source base.

In order to focus the binocular, which is set by commercial standards for infinity, upon the aerial image, two small additions were made. First, plus spheres of 3 D. power were placed before the objectives of the binocular. This addition was necessary in order to bring the focus of the instrument to one-third meter. Second, the parallel visual axes of the binocular were realigned to coincide with the aerial image. This was accomplished in experimental models by placing a 6 D. base-in prism before one of the objectives. However, these two additions, the plus spheres and the base-in prisms, may be combined by using segments of properly decentered plus spheres, or, for most ideal image visualization, by placing one large achromatic plus lens over both objectives.

The binocular is secured to the light source base by a movable plastic mount which firmly grasps the pivot hinge of the binocular, thus allowing the oculars to be

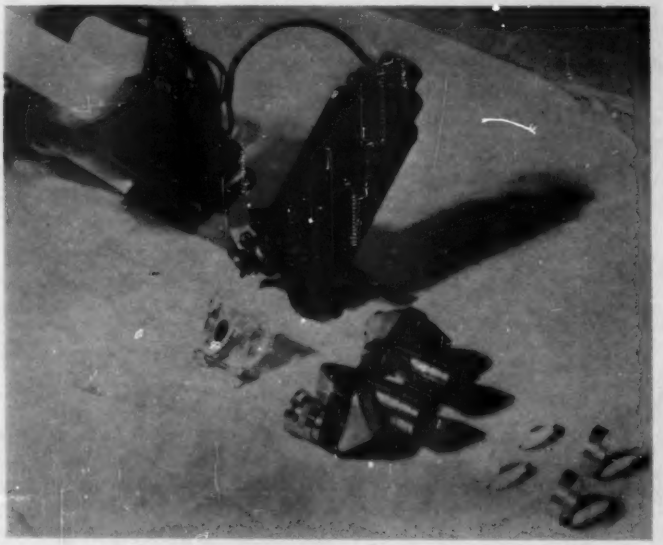

Fig. 2.—The unassembled, indirect ophthalmoscope showing, from left to right, the headband, the light source, plastic mount, binocular, decentered plus lenses, and the retaining rings.

manipulated and varied to conform to any interpupillary distance. The plastic mount may be loosened to permit the binocular to be quickly removed or replaced by other variously powered instruments.

Use and Features

The use of this indirect ophthalmoscopic arrangement differs only slightly from that of the conventional model. The convex lens is placed, as usual, several inches from the patient's eye. The observer then moves his head until the focus of his binocular coincides with the aerial image. The depth of field permitted by this optical arrangement is approximately one and one-half inches, and therefore, the head position assumed need not be critical.

The advantages gained in this modified indirect ophthalmoscope are the following:

1. Greater magnification of the aerial image is obtained without sacrifice of field. The linear field of the retina visualized with the binocular modification is *exactly* the same as that seen with the conventional indirect ophthalmoscope.

2. The stereoscopic effect is increased more than tenfold with the new modification. This increased perception of depth becomes extremely useful when attempting to ascertain small changes in the topography of the fundus. Flat detachments of the retina, early inflammatory foci, and choroidal tumors are among those lesions more easily diagnosed and defined in terms of extent and depth of the destructive process.

3. With the amount of magnification provided by this binocular attachment, the instrument has useful applications outside of fundoscopy. It can be employed whenever magnification and bright illumination would be helpful, such as in the external examination of the lids, conjunctiva, and cornea, and possibly in surgery of the anterior segment.

Summary

In summary, a modification of the Schepens-AO indirect ophthalmoscope has been presented which combines the advantageous features of greater image magnification, large fundus field, increased stereoscopic effect, and general adaptability in fields of direct visualization.

243 Charles St. (14).

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Oral Urea as an Osmotic Ocular Hypotensive Agent

MILES A. GALIN, M.D.; FUTABA AIZAWA, M.D., and JOHN M. McLEAN, M.D., New York

The use of osmotic agents in the treatment of all types of glaucoma has recently received impetus from the introduction of urea into clinical ophthalmology.[1] The theoretical superiority of this drug as compared to previously used osmotic solutions has been well substantiated. Its low molecular weight, relatively poor ocular penetrance, and inertness make it eminently successful as an ocular hypotensive agent. However, though the effects of oral urea have been reported,[2] most studies have dealt with the intravenous route of administration. Furthermore, no discussion has appeared relating intraocular pressure to oral urea administration. Consequently, with this latter thought in mind, the present study was undertaken.

Materials and Methods

Patients were obtained from the glaucoma clinic of The New York Hospital. Early pilot experiments revealed that absorption of the material was excellent, and that high blood levels could be obtained. A dosage level of 1.5 gm. per kilogram was utilized. The following routine was followed on all patients. Blood was obtained for subsequent analysis of freezing point depression and of urea nitrogen. Tonography was performed; and shortly thereafter, a 50% solution of lyophilized, ammonia-free urea dissolved in cherry syrup, and cooled with cracked ice, was administered. Most patients found the solution quite bitter, but were capable of consuming all of it. For comparison with the baseline studies, serial tonographic tracings were obtained, as well as blood samples at appropriate intervals. Urine was obtained from all patients and tested for the presence of hemoglobin.

		UREA 1.5g./kg. p.o.	
		BEFORE	AFTER
MILLIOSMOLES		296	324
B.U.N.		15	70
P_o	R.E.	29	14
	L.E.	30	18

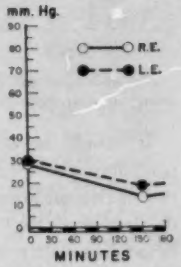

Fig. 1.—Man of 70 with open-angle glaucoma—effect of oral urea on intraocular pressure.

Results

Twenty patients with glaucoma have been studied. The results reported herein deal with five consecutive, unselected cases involving various types and stages of glaucoma. These illustrative results are typical of those obtained in all patients thus far treated with oral urea. A table and graph summarizing the pertinent data for each case follows in Figures 1-5.

		UREA 1.5g./kg. p.o.	
		BEFORE	AFTER
MILLIOSMOLES		286	309
B.U.N.		6	64
P_o	R.E.	36	15
	L.E.	28	11

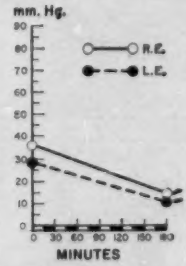

Fig. 2.—Man of 26 with congenital glaucoma—effect of oral urea on intraocular pressure.

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From the Department of Surgery (Ophthalmology), New York Hospital-Cornell Medical Center.

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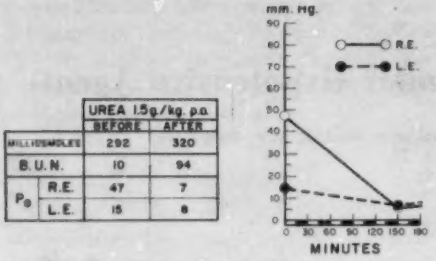
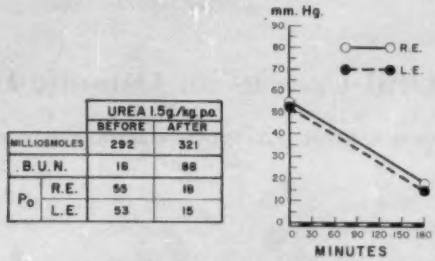


		UREA 1.5g./kg. p.o.	
		BEFORE	AFTER
MILLIOSMOLES		292	320
B.U.N.		10	94
P_o	R.E.	47	7
	L.E.	15	8

Fig. 3.—Man of 55 with open-angle glaucoma—effect of oral urea on intraocular pressure.

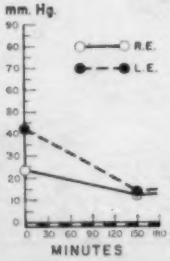


		UREA 1.5g./kg. p.o.	
		BEFORE	AFTER
MILLIOSMOLES		292	321
B.U.N.		18	88
P_o	R.E.	55	18
	L.E.	53	15

Fig. 5.—Woman of 73 with advanced open-angle glaucoma—effect of oral urea on intraocular pressure.

It should be noted that reduction in intraocular pressure is usually obtained between two and one-half to three hours from the ingestion of the urea. At this time the blood osmolality has reached its maximum, and corresponds linearly to the ocular pressure response. For the sake of conciseness, the graphs are not extended to include time intervals following the maximal hypotensive effect. However, the intraocular tension reaches the pretreatment level in about seven hours. The blood urea nitrogen and blood osmolality are normal within 36 hours. Urinary hemoglobin has not been found in any patient.

The nature of the patient's glaucoma in no way influences the osmotic response. However, as has been discussed elsewhere,[1] in secondary glaucoma the osmotic gradient is reduced, and the return to original hypertensive levels is more rapid than in the other types of glaucoma. No effort was made to study the effect of oral urea on acute glaucoma, as the time lag in response was considered undesirable.



		UREA 1.5g./kg. p.o.	
		BEFORE	AFTER
MILLIOSMOLES		288	323
B.U.N.		18	97
P_o	R.E.	23	13
	L.E.	42	14

Fig. 4.—Woman of 53 with R.E. open-angle glaucoma and L.E. aphakic glaucoma—effect of oral urea on intraocular pressure.

Comment

Intravenous urea has proved to be a truly remarkable agent for the reduction of intraocular pressure. Marked increase in blood osmolality is obtained, and hypotony is induced in about thirty to forty-five minutes.[1] However, for such conditions as pseudotumor cerebri, hyphema with imminent blood staining, and central retinal artery occlusion etc., prolonged rather than rapid hypotony is desired. Oral urea would seem to be efficacious under such circumstances.

The present study clearly indicates that urea is effective orally. With respect to osmolality changes, the dosage schedule of 1.5 gm. per kilogram orally is about equivalent to 1 gm. per kilogram intravenously.[1] We are presently investigating the prolonged use of oral urea as an ocular hypotensive agent. The palatability of the various oral preparations is the present limiting factor.

Summary

Urea is an effective agent for the reduction of intraocular pressure when administered orally at a dosage level of 1.5 gm. per kilogram.

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Annual Reviews

Glaucoma, 1958-1959

PAUL A. CHANDLER, M.D., Boston

I. Incidence

Reports on mass screening by tonometry show an incidence of glaucoma from 2%[90] to 4%.[48] Leydhecker et al.[63] in a mass screening of 10,000 people (19,880 eyes) found a medium Schiøtz tension value in normal eyes of 5.5/5.5. He considered that any value over 4/5.5 should arouse a suspicion of glaucoma, and that a value of 3/5.5 should be regarded as pathological. Andersen,[3] in a Danish town of 35,900 inhabitants, found 58 cases of primary glaucoma. The incidence steadily rose with each decade of life after 40, with the highest incidence, 27 per thousand, after the age of 85. In a study in Great Britain[104] cases of angle closure glaucoma outnumbered those of open angle glaucoma. In most reports, open angle glaucoma has been found to be considerably more common than angle closure glaucoma. Simmons[102] has given an excellent review of the literature on the incidence of glaucoma with regard to geographic distribution, seasonal distribution, age, sex, ethnic and race distribution, the effect of social and environmental factors, heredity, emotions, refractive errors, general body constitution, nutrition, and allergen or toxic sensitivity. He has suggested methods for the epidemiologic investigation of the disease.

II. Diagnosis

Provocative tests as an aid in early diagnosis of glaucoma have been discussed by several authors.[18,32,77,89,114] Tornquist found that the dark room test caused a rise of 8 mm. or more in 38% of unoperated but proven cases of angle closure glaucoma, in 14% of normal fellow eyes in patients with angle closure glaucoma in the other eye, and in 3% of eyes with a shallow anterior chamber but no known glaucoma. Miron et al. found changes in the light sense in all chronic and acute glaucoma. Primrose feels that the Priscal test raises pressure by increasing aqueous production. Weekers et al.[117] in a long outline of the mechanism of all forms of glaucoma both primary and secondary conclude that accurate tonometry is still the best method of diagnosing glaucoma. A bulbar compressor has been designed by Berens and Tolman.[11] With this instrument a pressure of 50 mm. Hg is applied to both eyes simultaneously through the lower lids for two minutes. Tension is measured before and after. They hope this may be of value in diagnosis and therapy. Certainly ophthalmoscopic examination of the optic disc and tonometry will in the long run establish the diagnosis in nearly all cases of open angle glaucoma.

III. Outflow Resistance

The anatomic distribution of resistance to outflow in normal human eyes post mortem has been examined by Grant[39] utilizing quantitative perfusion to measure facility of outflow before and after dissection of the trabecular meshwork (trabeculotomy or trabeculectomy). In a series of 16 eyes he has consistently found an increase in facility of outflow when an opening was made from the anterior chamber to Schlemm's canal, and calculates that the trabecular meshwork is normally responsible for approximately 75% of the total resistance to

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outflow. These measurements were made with the lens-iris diaphragm relaxed and the anterior chamber at normal depth. Presumably measurements can be made on the distribution of resistance in open angle glaucomatous eyes by the same technique, and comparison of normal and glaucomatous eyes should indicate the location of the abnormal resistance. For future refinement of this technique, it will of course be necessary eventually to make measurements and observations more immediately post mortem and also in vivo to minimize the possibility of confusion from postmortem changes. This study suggest the clinical possibility of improving facility of outflow in open angle glaucoma by trabeculotomy or trabeculectomy, particularly if in glaucomatous eyes the resistance to flow through the trabecular meshwork proves to be even greater than in the normal. If this proves to be true, and it seems altogether probable that it will be, it opens up the possibility of some form of surgery on the meshwork similar to goniotomy. An opening could be made into Schlemm's canal, but there is always the problem of healing with scar tissue formation.

Ericson[28] studied the 24 hour tension curve in normal individuals by means of a perilimbal suction cup after Rosengren, with a 50 mm. Hg vacuum obstructing the outflow channels. Using Friedenwald's 1955 tables, he was able to estimate the rate of aqueous flow. The aqueous humor inflow was studied by this method every four hours day and night for three days and nights on 50 subjects. During the day no change in aqueous humor inflow was found. Between 8 P.M. and midnight there was considerable reduction in aqueous flow. From midnight to 4 A.M. the flow was constant. From 4 to 8 A.M. there was considerable increase in flow as compared to the day level. He measured 10 subjects who worked at night. Similar variations were found in these cases but were less pronounced. He found intraocular pressure and inflow at a constant level between 8 A.M. and noon. Intraocular pressure decreased from a day pressure of 17 mm. to 14 mm. at midnight. There was no corresponding reduction in inflow during this time. At 8 P.M. and midnight the reduction in pressure was small but there was considerable reduction in aqueous inflow. From midnight to 4 A.M. there was an increase in intraocular pressure despite no significant change in inflow. From 4 to 8 A.M. both factors increased, the inflow increasing much more than the intraocular pressure. This could be explained by a change in outflow resistance as has been shown by tonography.

Linnér,[65] using the suction cup method, studied the rate of aqueous flow in individuals with and without cataract. He found the rate of flow definitely lower in cataractous eyes than in those without cataract. A vasodilating drug, Hexanicit, was tried and it appeared to increase the rate of aqueous flow. He conjectures as to its possible use in the medical treatment of cataract. Becker[7] employed tonography as an aid in prognosis in open angle glaucoma. Two hundred fifty eyes were studied in which the pressure was normalized by miotic treatment (Becker considers 20 mm. Hg a safer level for normalization than 24 mm. Hg). In eyes in which facility of outflow was normal under miotic treatment, he felt that the prognosis for future tension control and retention of the field was better than in eyes with a reduced outflow in spite of normalization of tension with miotics. This study indicates that with patients in whom the facility of outflow is still low, more intensive miotic treatment is indicated; secretory suppressors should be used and there should be a closer follow up. This work should be very helpful. Eyes with a poor outflow facility might be expected to be more unstable from the standpoint of tension, since a relatively small increase in rate of aqueous production would be reflected in a considerable rise in tension. However, in making a prognosis in open angle glaucoma, one cannot fail to take into

consideration the appearance of the optic disc and the extent of field loss if present. All other things being equal, an eye with a normal disc is well known to withstand increased pressure much better than a cupped disc.

In a study of angle closure glaucoma, Becker and Thompson [10] found that if the coefficient of facility of outflow was 0.15 or better, peripheral iridectomy would control the glaucoma and that filtering operations should be reserved for eyes with a coefficient of facility of outflow of 0.10 or less. They also measured facility of outflow in normal eyes before and after mydriasis with 5% Euphthalmine. They felt that a decrease in outflow facility of more than 25% after mydriasis was a valuable method of recognizing the capacity for angle closure in eyes with narrow angles. Becker and Thompson [10] found no failures with peripheral iridectomy in angle closure glaucoma in 47 eyes where the coefficient of facility of outflow was over 0.20. There was one failure in 71 eyes where the C was over 0.15. Peripheral iridectomy failed to control the glaucoma in 10 of 13 eyes where C was less than 0.10. In 14 eyes in which peripheral iridectomy failed to control the glaucoma, subsequent iridencleisis was successful in all but 3.

There is no doubt, as these studies demonstrate, that accurate tonography can be of considerable value in the choice of operation in angle closure glaucoma.

Constant pressure tonography was studied by Prijot [58] and Moses.[79] Prijot's results were the same as Grant's in normal eyes, but he found a greater resistance to outflow in open angle glaucoma than found by Grant's method. Moses felt that constant pressure tonography could not be recommended for routine use on account of its complexity. Heinrich [44] studied 97 cases pre and postoperatively by tonography. Of 11 cases showing progressive field loss postoperatively, he found in 8 a further decrease in outflow facility. His best surgical results were obtained with iridencleisis. Further studies on tonography were reported by Merte [75] and Yoshitoshi.[87] Armaly and Burian [4] found an increase in facility of outflow during accommodation in five young normal individuals. They could not be sure whether the steeper slope in the tonogram was due to a greater facility of outflow, a reduction in aqueous production, or in intraocular blood volume.

Depner [20] has given an excellent, detailed and thorough survey of the whole subject of tonography with a thoughtful analysis of the many factors to be considered in the application and interpretation of tonography. The pertinent literature is also surveyed. He has emphasized the research and investigative aspects of tonography. This is a valuable paper for those interested in tonography.

IV. Tonometry

For the purpose of increasing the accuracy of tonometry and tonography, McBain [72] has continued his careful analysis of pressure-volume relationships in enucleated human eyes. He has obtained additional data by repeatedly injecting finite measured volumes of salt solution through a cannula into the eye and continuously recording the resultant step-wise changes in intraocular pressure by means of an electronic strain gauge. His analysis of these data indicate that scleral rigidity is more complex than was pictured by Friedenwald when he constructed the standard calibration tables for the Schiøtz tonometer. He has worked out formulas to describe the pressure-volume relationship more precisely, and, with further measurements to be made on the volume of corneal indentation which is induced by the tonometer, these should eventually benefit clinical tonometry and tonography.

Moses and Hahn [80] found that the P_o value obtained with the 5.5 and 7.5 gm. weights according to the 1955 Friedenwald tables were inconsistent with a scale reading below 3, the 7.5 gm. weight giving the lower estimate of P_o. They felt that the

size of the footplate hole influences the scale reading in a range of 3 or less, the smaller hole giving the greater scale reading. By direct observation through a plastic footplate, they found that at pressures corresponding to a scale reading of 2 or less the cornea bulged up into the gap between footplate and plunger. Jackson [49] compared the Schiøtz X tonometer with the Schiøtz weighted tonometer in a large series of normal and glaucomatous eyes and found very little difference in the results.

V. Perimetry

Sanna [94] gave examples of alterations of the visual fields in cases of glaucoma due to the simultaneous presence of other ocular disease. This is a pertinent and neglected subject. Eyes with glaucoma may indeed have field defects due to a variety of causes, including arterial occlusion, retinoschisis, retinal detachment, and chorioretinitis. One cannot accept a field defect as due to glaucoma unless it is consistent with the appearance of the optic disc. Campbell and Ritter [15] report on flicker perimetry in chronic simple glaucoma. They found several cases in which the field was normal as measured by conventional perimetry but defects were demonstrated by flicker perimetry. Where a field defect was found by ordinary perimetry it was usually more marked with flicker perimetry. They conclude that flicker perimetry is valuable, especially in early glaucoma.

Harrington and Flocks [41] reported on the multiple-pattern method of visual field examination. The literature is reviewed and they report their own further experience with the method. New patterns are described. The method has proved its value in the hands of many ophthalmologists. In glaucoma perimetry the first consideration is the ophthalmoscopic appearance of the optic disc. One cannot find field defects due to glaucoma unless there are ophthalmoscopically visible changes on the disc.

VI. Pathology

Wolter [118] presented the histologic findings in the angle in the normal eyes of a 2-year-old child who had an eye with an epithelial cyst in the anterior chamber, and an eye with a shallow anterior chamber and a closed angle after cataract surgery. The tissues were stained with the threefold and unreduced methods of the silver carbonate techniques of Hortega. In the child there was vacuolization of the corneal endothelium but not of the endothelium of the trabecular meshwork. In the eye with the epithelial cyst, endothelial cells were absent in the meshwork. In the closed angle there was degeneration of collagen in the outer layers of the corneoscleral meshwork and a diminution in the number of endothelial cells. Nerve fibers were demonstrated in the meshwork especially in the layer adjacent to Schlemm's canal. He found many openings in the meshwork approximately 10μ in diameter. He believes that aqueous travels through the meshwork in a series of thin walled intercommunicating channels.

Teng et al.[111] examined histologically six eyes with successful filtering operations. They conclude that contact with aqueous causes degeneration of collagen. They found that in some eyes aqueous goes directly through the bleb on account of collagen degeneration in the subconjunctival spaces. In other eyes there was degeneration of collagen tissue around blood vessels, especially capillaries, causing perivascular degeneration and opening of new channels. They found that direct new recanalization occurs in cases where the scleral wound is close to the trabecular region and new endothelial lined channels form.

Kornzweig and Schneider [55] studied the pathology of the angle of the anterior chamber in 11 eyes of 7 patients known to have primary open angle glaucoma. They found thickening and sclerosis of the trabeculae, narrowing or obliteration of the intertrabecular spaces, variation in the patency of Schlemm's canal, pigment granules and cells in varying amounts in the

intertrabecular spaces, and proliferation of endothelium causing occlusion of Schlemm's canal.

Rohen and Unger [93] in a histologic study of the trabecular meshwork found proliferation of the endothelium in both primary and secondary glaucoma. In inflammatory reactions the endothelial cells increased in size and were sometimes desquamated. They also found thickening of the subendothelial basement membrane in various cases of glaucoma, with an extreme degree in absolute glaucoma.

Flocks,[29] in an excellent and comprehensive article, describes a pathologic study of the trabecular meshwork in 16 eyes with primary open angle glaucoma and in 11 eyes of secondary glaucoma with an open angle. The author describes the various pathologic findings and concludes that many of the pathologic changes found were due to the increased tension per se. From this study emerges a new theory of open angle glaucoma. He believes that a vulnerable corneoscleral meshwork is inherited. This vulnerable meshwork is injured by a tension lower than in individuals with a normal meshwork. More and more damage to the meshwork occurs and there is hence a gradual decrease in the facility of outflow. This paper should be read in the original.

VII. Medical Treatment

Dichlorphenamide in a dose somewhat less than half that of acetazolamide (Diamox) has been found to have a comparable effect in lowering intraocular pressure in glaucoma.[36,46,97] Side effects are similar to those of acetazolamide. Some patients tolerate one drug and not the other. Chlorothiazide (Diuril) was found to have no effect on intraocular pressure, and sulocarbilate had some pressure lowering effect but only in doses 3 to 6 times that of acetazolamide.[46] Acetazolamide is generally found to reduce the formation of aqueous about 50% and has proved generally very useful in most forms of glaucoma.[1,8,19,38] Epinephrine bitartrate, a valuable drug in the treatment of open angle glaucoma, has been found to cause a further 30% reduction in aqueous production in addition to that obtained with acetazolamide, and hence, the effect of the two drugs is additive.[8] Epinephrine bitartrate is gradually being accepted more and more widely as an adjunct to miotics in the treatment of open angle glaucoma. It is an extremely valuable drug. In some eyes epinephrine bitartrate and phenylephrine caused a rise in pressure lasting several hours which was demonstrated by tonography to be due to an increased resistance to outflow. The usual effect of lowering of intraocular pressure was not due to an increased facility of outflow as demonstrated by tonography.[61]

The effects of echothiophate (Phospholine) iodide were studied by Becker et al.[9] In normal eyes this drug used in a concentration of 0.25% once a day caused an increase in the facility of outflow of 66%. Side-effects included extreme miosis, ciliary and conjunctival injection, blurred vision, and eye and brow pain. The miosis persisted for 12 to 14 days, the other side effects usually disappearing in 5 days. The drug was tried on 106 eyes with both primary and secondary glaucoma uncontrolled by medical therapy. The facility of outflow improved 25% or more in 72% of eyes, 50% or more in 58%, 100% or more in 40%. Tension was lowered to 20 mm. in 50% of eyes in which the tension was previously uncontrolled. A subconjunctival injection of pyridine-2-aldoxime methiodide (P_2AM) reversed the effect of the drug in both normal and glaucomatous eyes. The side effects observed in normal eyes were also observed in cases of glaucoma more or less in proportion to the effect on tension and facility of outflow. Iris cysts were seen when the drug was used in children. Several patients had diarrhea. In three patients severe iritis developed three to five days after starting treatment.

Turtz and Turtz [112] reported a case of toxicity due to acetazolamide. In January, 1957, the patient was given 125 mg. of

acetazolamide t.i.d. In May, 1957, the patient developed exfoliative dermatitis and the white blood count was found to be 3,050. When the drug was discontinued the dermatitis cleared in a few days; one week after discontinuing treatment the white count had risen to 7,950. Mackie[68] presents arguments supporting the "vascular" theory of glaucoma. In 50% of patients in which the glaucoma was uncontrolled with miotics the tension was brought under control when hydrolazine hydrochloride (Apresoline) and Hydergine* were given. He stresses the psychologic aspects in managing the patient with glaucoma.

VIII. Surgical Treatment

Three new operations have been described utilizing cautery of the wound edges to produce shrinkage of the sclera. In Scheie's procedure,[95,96] after preparing a conjunctival flap as for a conventional filtering operation, the sclera is cauterized with the actual cautery along a line close to and parallel with the limbus. A right angled incision is made through the sclera in this cauterized area and the lips of the wound are touched with the cautery, causing scleral shrinking and gaping of the wound. A peripheral iridectomy is done. He reported results on 28 eyes with angle closure glaucoma and 43 with open angle glaucoma. Tension was controlled at normal levels in 48 eyes, uncontrolled in 10. In 15 eyes there was hypotony—tension 10 or less. Complications of delayed reformation of the anterior chamber, hyphemia, persistent hypotony, were comparable to those after trephing or iridencleisis. The operation was successful in one of three eyes with congenital glaucoma uncontrolled by previous operation. He feels that the procedure is particularly applicable to cases of neglected angle closure glaucoma.

Alvis[2] describes an operation in which under a conjunctival flap a radial incision 3 mm. long is made out from the limbus. A cyclodialysis spatula is passed to either side to separate the scleral spur. The scleral wound edges are then touched with cautery to cause gaping. In several eyes a filtering scar developed. In a small series of cases he feels the results were comparable to those of other filtering operations. Malbran and Malbran[69] described an operation similar to that described by Scheie, but using diathermy instead of thermal cautery to cause shrinkage of the wound edges. In 50 cases the result was considered good, tension under 23.4 mm. in 41. The tension was borderline 25 to 29 in six cases and uncontrolled in three. Delayed reformation of the anterior chamber was seen in three cases. A considerable experience over a long period of time will be necessary in order to properly evaluate these operations and determine whether or not they are superior to the older procedures.

There have been numerous reports on the use of cyclodiathermy.[31,56,58,66,67,76,100,113] It is difficult to compare the results of various reports since the period of follow-up was often inadequate. There are wide differences of opinion as to the effectiveness of the operation, ranging from enthusiastic reports to very pessimistic ones. The general feeling seems to be that the results are often not permanent and the operation has to be repeated. The fact that cyclodiathermy is still not widely used as a primary procedure in glaucoma attests the fact that the long term results are inferior to the conventional procedures. It is certainly a valuable addition to our armamentarium in glaucoma surgery, but probably its principal usefulness will continue to be in hemorrhagic glaucoma and in cases of primary glaucoma where other operations have failed. Combined nonperforating cyclodiathermy, and limited cyclodialysis is reported by Cavka.[17] In cases with an adequate follow-up there was a fairly good per cent of tension control. No details are given concerning the presence or absence of a cleft.

*A pharmaceutical combination of dihydroergocornine, dihydroergocristine, and dihydroergocryptine.

Cohen et al.[23] reported a successful result in 8 of 10 cases in colored patients in which iridencleisis with sclerectomy was followed by beta radiation; 2,100 rep. was given at the end of operation and repeated at weekly intervals for a total dosage of 6,300 rep.

Nemetz and Papapanos[81] reported the results of operation in 276 cases of advanced glaucoma in which the field was contracted to within 5 degrees of fixation in at least one quadrant. In two-thirds of the cases vision was the same or one line less postoperatively. In 212 eyes with visual acuity more than 6/60, 6% became blind after operation. In 64 eyes with visual acuity less than 6/60, 64% went blind. In nine eyes with two or more operations all became blind. The authors advise operation in all cases with vision better than 6/60, medical treatment if vision is 6/60 or less. It is difficult to draw conclusions from these data. A scotoma within 5 degrees of fixation in one area with three-fourths of the field intact is quite a different situation from an isolated small central field. If, postoperatively, tension is maintained at a low level, 7/5.5 or less, most eyes will maintain their field over a long period of time, whereas if postoperatively tension ranges from 5/5.5 to 3/5.5 many eyes will progressively lose field.

Hartmann and Haye[42] report on Beren's cycloelectrolysis. They conclude that it is an operation easy to perform, not dangerous, easy to repeat, and can be tried when other operations have failed. Best results were obtained in glaucoma following cataract extraction. It was found effective in acute glaucoma, less so in chronic glaucoma. They report that it always relieves pain in hemorrhagic glaucoma though tension is uncontrolled in half the cases. Golovine[35] reported success in nine cases of very intractable glaucoma by trephining the sclera 6 or 7 mm. from the limbus under a conjunctival flap and letting out vitreous. Two cases of malignant glaucoma with blocking of the scleral opening by the lens were relieved by lens extraction.[91] Cross[24] described a case of malignant glaucoma following iridectomy. Following posterior sclerotomy, cyclodialysis, and air injection into the anterior chamber, convalescence was uneventful. In the second eye peripheral iridectomy and posterior sclerotomy were done, but a malignant course followed. The glaucoma in this eye was also relieved by the same procedure done on the first eye.

Riise[92] studied the records of 1,057 eyes with glaucoma simplex on which iridencleisis had been done between 1930 and 1956. There were 100 eyes with adequate field examinations and normal tension over a period of five years. In a group with normal or slightly constricted fields, after eight years there was loss of 7% of the field. In a group with loss of more than half the field at the time of operation, the results were much poorer; 15 of 39 eyes lost all useful vision. All eyes lost visual acuity. Riise's observation that eyes with normal or slightly constricted fields showed very little further loss over a considerable period is an important one. It has become increasingly evident in clinical practice that eyes with little or no cupping and little or no field loss withstand increased pressure much better than eyes with considerable cupping and field loss. Results of filtering operations are reported by other authors.[53,40,120] Late infection after filtering operations was reported by Kalt and Loisillier.[51] There were 13 cases of late infection in 464 operations. The incidence was 3.23% after trephining, 1.6% after the LaGrange operation, and 1.4% after iridencleisis. Results of treatment were good if no hypopyon was present but poor if there was hypopyon. Sugar and Zekman[109] reported 22 eyes infected after trephining, 2 after iridencleisis in clinic patients. In private practice in 48 trephine operations there was 4% late infection. In 66 iridencleisis operations the incidence was 1.5%.

In filtering operations Stanworth[106] suggests that some failures are due to conjunctival fibrosis rather than closure of the scleral opening and recommends excision of tenons capsule near the trephine opening.

This is particularly important in young people where there is considerably more subconjunctival tissue than in older individuals.

McCulloch [73] describes a method of reopening localized blebs after filtering operations. A cataract knife is passed under the conjunctiva 10 mm. from the bleb. It is pushed through and through the bleb and the cut is enlarged upwards subconjunctivally. In 9 of 12 cases the operation was successful. This is certainly a very good percentage of success with a much less traumatic procedure than another filtering operation.

IX. Congenital Glaucoma

Burian [14] believes there are two types of developmental glaucoma. In one type the glaucoma is due to faulty splitting of mesodermal tissue in the angle. There is often a prominent Swalbe's line, the iris root encroaches on the meshwork, there are prominent pectinate fibers, and Schlemm's canal is normal. He feels that goniotomy is suitable for this type. In the second type the iris encroaches on the meshwork more markedly, there are no prominent pectinate fibers or Swalbe's line, and Schlemm's canal is abnormal or absent. Goniopuncture or some other filtering operation is recommended for this type.

Paufique [83] supports entirely Barkan's views of the pathogenesis of congenital glaucoma. He advises goniotomy when this is possible. When it is not possible, as in advanced cases with opaque corneas and enlarged globes, he advises retrociliary diathermy or angiodiathermy at the medial rectus insertion plus cyclodialysis at the same site.

Shirley [101] reported two cases of glaucoma in young people. Both showed abnormal congenital tissue in the angle. In the first patient, age 18, the glaucoma was unilateral and was controlled by two goniotomy and goniopuncture procedures. In the second case, age 32, the glaucoma was controlled by iridencleisis. Priegnitz [87] reported two cases of juvenile glaucoma. The first patient was a 19-year-old boy with wide angles showing the typical changes of congenital glaucoma. The discs were cupped, tension 30 to 40. The glaucoma was controlled by miotics. Patients in the teens and twenties with glaucoma frequently show the same appearance in the angle on gonioscopic examination commonly seen in young children with glaucoma. One wonders if goniotomy should not be employed more frequently in these cases. The second patient was 22 D. myopic with spherophakia. The anterior chambers were shallow and the angles narrow. Tension rose with the dark room test and with miotics but fell when mydriatics were used. The glaucoma was relieved by removing the lenses and it was then apparent that the previous myopia had been all lenticular. Maumenee [70] reported on the pathologic examination of six eyes with recent congenital glaucoma, two eyes with moderately advanced glaucoma, and many eyes with advanced congenital glaucoma. He found in all cases a failure of separation of the iris and ciliary muscle from the trabecular fibers and an abnormally extensive insertion of longitudinal and circular muscle fibers into the trabecular fibers. The ciliary body was pulled centrally. In cases with successful goniotomy the meshwork was intact, but the ciliary muscle and iris were detached from the trabecular fibers. He concludes that the abnormal attachment of the ciliary body muscles to the meshwork by their contraction decrease the pore size in the meshwork and thus cause increased resistance to outflow. In seven of eight eyes with persistent hyperplastic vitreous the angle showed changes similar to those in eyes with congenital glaucoma. Shaffer [99] has given a careful exposition, with photographs, of the proper techniques in goniotomy.

X. Secondary Glaucoma

Gärtner [32] has given a detailed report on two cases of Marchesani's syndrome. He found that tension was increased by miotics,

lowered by mydriasis. He believes the cause of the glaucoma is twofold, an abnormal development of the ciliary muscle which causes a narrow chamber angle, and pupillary block. He advises lens extraction and treatment of any residual glaucoma by miotics. After lens extraction he found the angles closed or very narrow, but there was considerable opening with miotic therapy.

Peterson [84] has made a careful study of a large series of patients with exfoliation, with and without glaucoma. He found a very good correlation between the amount of pigment in the meshwork and the severity of the glaucoma. In eyes with exfoliation in one eye only, there was more pigment in the meshwork in the eye with exfoliation than in the fellow normal eye. In cases with bilateral exfoliation with glaucoma is only one eye, the glaucomatous eye showed much more pigment in the meshwork than in the eye without glaucoma. In patients with exfoliation but without glaucoma which were observed over a period of several years, there was very little pigment in the meshwork. This careful study lends weight to the theory that it is the accumulation of pigment in the angle which is the important factor in obstruction to outflow in capsular glaucoma. In eyes with exfoliation and glaucoma but no unusual accumulation of pigment in the meshwork it seems probable that the glaucoma is primary and bears no relation to the exfoliation. In patients known to have exfoliation, who are being followed regularly with the possibility of glaucoma in mind, the prognosis remains good as long as there is little accumulation of pigment in the meshwork. Henderson and Schneider [45] reported the ocular findings in 17 cases of carotid cavernous fistula. Among other findings, secondary glaucoma was present in seven cases. In one of these, the glaucoma was controlled by miotics, in three cases by surgical treatment of the fistula, but in three cases blindness followed operation.

Chandler [21] has discussed the problems in the diagnosis and treatment of lens induced uveitis and glaucoma of various types. Case reports are given illustrating lens induced uveitis and glaucoma from hypermature cataract in situ, completely dislocated hypermature cataract, advanced immature cataract, traumatic cataract, and in cases after extracapsular extraction. In all cases removal of the lens or of residual lens matter resulted in subsidence of the inflammation. The glaucoma was also relieved except in cases with residual peripheral anterior synechias. He points out that prompt lens extraction is indicated if the lens is intact and that in the cases with residual lens matter, removal of this lens matter results in prompt subsidence of the inflammatory signs. Delay in removing lens matter results in irreversible changes in the eye such as persistent glaucoma, corneal edema, retinal detachment, and cystic macular degeneration. Syed [110] concurs that in cases of hypermature cataract and glaucoma lens extraction is indicated.

Secondary glaucoma following occlusion of the central retinal artery has been the subject of two reports.[85,110] The glaucoma usually developed within nine weeks. In all reported cases the central vein showed changes but was not occluded in the sections examined. The changes found in the angle were similar to those usually seen in cases of hemorrhagic glaucoma following occlusion of the central vein. In these cases one must consider the possibility that the vein may have been occluded beyond the area examined.

Heer [43] attributes the ectropion uveae in hemorrhagic glaucoma to a new formed membrane on the surface of the iris. He observed gonioscopically in several eyes an exudative process on the anterior surface of the iris which led to a contracting membrane and closure of the angle. Schrader and Medler [98] reported on a large series of cases of central vein occlusion. Secondary glaucoma was not seen in any patient under 50 years of age. Of the patients with glaucoma, in seven the tension was only slightly elevated and was controlled by miotics. This observation, plus the report that in 12 patients iridencleisis was suc-

cessfully employed, must raise the question as to whether these were instances of true hemorrhagic glaucoma. The possibility of primary glaucoma unassociated with the central vein occlusion is shown by Posner,[86] who reported typical primary angle closure glaucoma in cases with retinal vein occlusion. He points out that hemorrhagic glaucoma cannot be diagnosed without observing neovascularization followed by peripheral anterior synechias. Gorin[37] emphasizes the fact that in hemorrhagic glaucoma acute iritis is always a factor. He rightly stresses the fact that miotics should not be used but rather mydriatics, cortisone, and acetazolamide. He reported two cases with relief of the pain following retrobulbar injection of procaine and Priscoline. Like Posner, he emphasizes the fact that the acute glaucoma seen after occlusion of the central vein may be primary angle closure glaucoma or due to the lens, and thus is not always true hemorrhagic glaucoma. These observations point up the fact that one cannot diagnose hemorrhagic glaucoma without finding the characteristic changes in the angle.

Jones[50] reviews four cases reported by various authors and reports one case of typical hemorrhagic glaucoma following intensive radiation. In his case cyclodiathermy was successful.

Hermann[47] reported on a syndrome in which there was microphthalmos, fundus changes suggestive of retinitis pigmentosa, cataract, and glaucoma. Cases were observed in four generations of one family. Blindness was common by the age of 30 due to cataract and glaucoma.

XI. Research

Karg et al.[52] perfused enucleated human eyes with latex microspheres of varying diameters. Particles 1.2μ in diameter passed through the eye without appreciable loss in concentration. There was some interference with the passage of particles 1.8μ and 3.μ in diameter and particles 5.6μ in diameter did not perfuse through. De Carvalho[18] studied changes in the blood aqueous barrier in rabbits as a result of tonography. He found that anterior chamber puncture in one eye resulted in increased permeability of the blood aqueous barrier in the other eye. There was increased permeability of the blood aqueous barrier immediately after tonography, but he feels that this does not prove that it exists during tonography. Lieb et al.[64] studied the effects of cervical sympathetic ganglionectomy on aqueous humor dynamics. On the first postoperative day average tension was 11.93 on the side of operation as compared to 27.31 on the normal side. The facility of outflow was about the same on both sides. The rate of flow on the side of operation was 0.63 cu. mm. per minute, and 3.64 cu. mm. per minute on the normal side. On the third postoperative day all values had returned to normal. By a series of equations McEwen[74] concludes that the increased resistance to outflow in glaucoma is probably due to a decrease in the diameter of the pores rather than a decrease in the number of functioning pores. He feels that the inner part of the trabecular meshwork offers little resistance to the passage of aqueous and that changes in the collector channels and aqueous veins are probably not important factors in the increased outflow resistance in glaucoma.

Barsky and Schimek[6] investigated the results of placing a strip of Gelfilm in cyclodialysis clefts in rabbits. In all cases there was considerable reaction and the cleft closed. They conclude that it cannot be recommended for use in human eyes. Berggren,[12] contrary to the reports of other authors, found in rabbits that the daily subcutaneous injection of 25 to 500 mg. of citral caused no elevation of intraocular pressure. Moi[78] reported perfusion experiments on human and rabbit eyes varying the perfusion pressure. He found that with a perfusion pressure increased up to 70 mm. the facility of outflow was essentially unchanged. The facility of outflow was slightly higher in vitro than in vivo. Miotics and mydriatics had no effect on facility of out-

flow. In an eye with retinal detachment and hypotonia there was a markedly increased facility of outflow. In one eye, when the lens was dislocated backward, there was a considerable increase in facility of outflow. Auricchio and Barany[5] studied rabbits in which experimental uveitis was produced in one eye. Outflow resistance was 114% higher in the eyes with uveitis. Episcleral venous pressure was 9.37 and 8.88 in the eyes with uveitis and the normal eyes, respectively. Average intraocular pressure was 13.1 in the eyes with uveitis as compared to 20.6 in the normal eyes. They felt that the low intraocular pressure and increased resistance to outflow was due to a suppression of aqueous formation.

Stepanik[107] reported on tonographic studies in connection with the water-drinking test in glaucoma simplex. In 58 cases of glaucoma simplex tonography was done before 30 and 60 minutes after water-drinking. In eyes with a negative water-drinking test there was a significant increase in resistance to outflow after 30 minutes. The minute volume of aqueous humor flow remained unchanged. In eyes with a positive water-drinking test the outflow resistance was unchanged and the minute volume of aqueous humor drainage was significantly raised after 30 minutes.

Campbell et al.[16] studied the effect of acetazolamide on plasma bicarbonate and on the electrolyte balance in relation to intraocular pressure. They found no direct association between the small reduction in plasma bicarbonate and the immediate fall in intraocular pressure after acetazolamide. The intraocular pressure appeared to behave independently of a low level of plasma bicarbonate. The pressure lowering effect of acetazolamide was therefore probably associated with some other aspect of its systemic action. If acidosis is induced, acetazolamide causes no fall in intraocular pressure. Decrease in sodium and chloride ions are probably of more significance than that of other ions.

Langham[59] found that methazolamide (Neptazane) was 2 to 3 times more active than an equal weight of acetazolamide in lowering intraocular pressure.

Gloster and Greaves[34] studied the effect of cervical sympathectomy on the ocular response to hypothalamic stimulation. In cats, stimulation of the hypothalamic region usually caused a fall in intraocular pressure in the ipsilateral eye, little if any in the contralateral eye. The fall in tension was abolished by cervical sympathectomy.

Maurice[71] described an experimental recording tonometer held in place by a headband and worn for 20 minutes continuously recording tension.

Flocks et al.[30] produced glaucoma experimentally in animals by placing a tight rubber band around the equator of the eye. The tension was initially quite high, gradually decreasing over periods of from 3 to 34 days. The average duration of increased tension was 14 days. The anterior chamber remained deep. Cupping and atrophy of the disc ensued.

Stone and Sears[108] studied the intraocular pressure in experimental cross circulation in rabbits. Having established cross circulation, a pressure of 100 mm. Hg was applied to the eye of one rabbit. In the second 10 minute period there was some fall in tension in the undisturbed animal. In controls without pressure, there was no fall in tension. In a group with transfusion of blood from an animal which had had compression of the eye, there was some fall in tension in the first 10 minute period in the animal receiving the transfusion. The result was negative if pressure had not been applied to the donor animal. These experiments suggest that there is a circulating factor possibly a hormone which regulates intraocular pressure, possibly a neurogenic factor.

Kornblueth et al.[54] studied the effect of anesthesia on intraocular pressure. All anesthetic agents caused a significant drop in tension ranging from 5.7 mm. Hg with ether to 7 mm. Hg with thiopental sodium. (Pentothal Sodium). The maximum decrease was in the stage of deep anesthesia. Tonography was done on several patients.

Thiopental sodium caused an increase in the coefficient of facility of outflow from 0.18 to 0.28. There was less increase with ether. Curare caused an average drop of 2.8 mm. in tension, and an 18% decrease in the coefficient of facility of outflow. The authors believe that the lowering of tension is via the hypophthalmos and believe that their studies suggest a central control of tension. Cohan,[22] by means of radiography, studied the outflow channels in rabbits; 0.2 cc. of a 50% solution of sodium diatrizoate (Hypaque sodium 50%, Winthrop Stearns) was used to replace the same volume of aqueous. X-ray photographs were taken 20 minutes later. From the ciliioscleral sinus, a cleft which communicates with the anterior chamber, numerous collector channels led to the deep scleral plexus. Two outflow channels originated from the plexus. First, communicating vessels passed through the sclera to emerge as aqueous veins. Second, anastomotic vessels extended to a plexus of veins in the ciliary muscle. This plexus communicated with the vortex vein system, and in addition, perforating vessels from it emerged on the scleral surface as laminary veins.

Törnquist and Broden[116] studied the chamber depth in 145 cases of simple glaucoma. In general no striking difference from the normal was found, but there was a tendency toward a slightly shallower chamber in glaucoma. They felt that inadvertently some cases of angle closure glaucoma might have been included. If, on the average, the anterior chamber is more shallow in glaucomatous than in normal eyes, it is not clear how it would favor the development of glaucoma. Törnquist[115] also studied the peripheral chamber depth in eyes with a generally shallow anterior both normal and glaucomatous. He found the angle narrower in the upper than in the lower part of the anterior chamber. In glaucomatous eyes the angle was narrower in proportion to the axial depth of the anterior chamber than in normal eyes. After peripheral iridectomy the values were about equal to those in normal eyes, suggesting that in glaucomatous eyes there is a certain degree of iris bombé, a fact that has been noted by many observers.

Dellaporta[25] devised a method of separating the corneoscleral meshwork from its bed as a part of cyclodialysis. The cyclodialysis spatula is twisted so that the blade is at a 45 degrees angle with the sclera. In enucleated human eyes, the meshwork was lifted off and Schlemm's canal exposed by this method in a high percentage of cases.

XII. Miscellaneous

Duke-Elder[27] states that an anatomically narrow angle is a prerequisite for acute angle closure glaucoma, and that iridectomy by establishing a free communication between anterior and posterior chamber cures the disease if done early. He believes that the actual closure is due to systemic factors; vasomotor instability which results in congestion of the ciliary body, and an outpouring of aqueous into the posterior chamber pushing the iris forward and closing the angle. He believes operation is always justified and even prophylactic peripheral iridectomy for the fellow eye. Outpouring of excess fluid into the posterior chamber could easily account for angle closure, but there is no evidence that such excess formation of aqueous does in fact occur.

Chalst et al.[20] point out that belladonna and belladonna-like drugs given for relief of gastrointestinal disorders and parkinsonism may have a bad effect on eyes with glaucoma or a predisposition to glaucoma. In eight subjects with normal eyes these drugs caused a mild increase in tension. The size of the pupil and accommodation were affected in most eyes. A synthetic antispasmodic drug, dicyclomine hydrochloride (Bentyl), did not cause an increase in tension and did not affect pupil size or accommodation in 37 normal patients and 7 glaucomatous patients.

Gartner and Billet[32] reported four cases of acute glaucoma developing within 48 hours of a general surgical procedure. The

cause was thought to be the atropine and scopolamine used preoperatively and the dilation of the pupil under deep anaesthesia, plus the predisposition of a narrow angle. They recommend 2% pilocarpine as a prophylaxis in such cases. This seems a simple and reasonable practice for routine use and would no doubt prevent many attacks of acute angle closure glaucoma.

Lasco and Nicolesco [60] discuss pseudoglaucoma which they define as atrophy and excavation of the disc without other evidence of glaucoma. Diseases which they state occasionally or rarely produce this condition are methyl alcohol poisoning, sclerosis of the internal carotid, intracranial or intraorbital tumors, temporal arteritis, systemic arteriosclerosis, and hypo- or hypertension.

Lerman [62] has given an excellent discussion of the mechanisms of the various forms of glaucoma both primary and secondary. He covers the subject of aqueous formation, tonography, possible homeostatic mechanisms in the control of intraocular pressure, primary glaucoma both from the standpoint of mechanism of production, as well as treatment both medical and surgical. Treatment of secondary glaucoma is also discussed.

Sloan [103] discusses the practical considerations in the management of glaucoma in office practice with regard to diagnosis, classification, treatment, and follow-up. This is a sound practical discussion for the practitioner of ophthalmology.

5 Bay State Rd. (15).

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News and Comment

ANNOUNCEMENTS

Caracas Program for Pan American Congress of Ophthalmology.—Space exploration as it may effect the eyes will lead off the scientific program of the sixth Pan American Congress of Ophthalmology, to be held in Caracas, Venezuela, Jan. 31-Feb. 6, 1960.

The tentative program, announced by Dr. Brittain F. Payne, New York, President of the Pan American Association of Ophthalmology, promises discussions on subjects ranging from genetics to autopsies, presented in symposia, round tables and free papers in two concurrent sessions throughout the week. Simultaneous translation of scientific papers is planned.

Brig. Gen. Victor A. Byrnes, U.S. Air Force, Washington, D.C., and Dr. David Schoch, Chicago, will preside at the session on space ophthalmology. Speakers who will consider the possible effects on the eye of various conditions in space flight will include: Capt. James L. Fuelling, U.S. Naval Air Station, Pensacola; Major William T. Sallee, U.S. Air Force; Drs. Albert D. Ruedemann Jr., Detroit, and Walter H. Benedict, Knoxville, Tenn., and Dr. Schoch.

In a symposium centered upon hereditary conditions of the head and neck that may affect the eyes, Dr. Harold F. Falls, University of Michigan Medical School, Ann Arbor, Mich., and Dr. Guillermo Pico, Santurce, Puerto Rico, will be joint chairman, and both will present papers. Dr. Max A. Finton, Kalamazoo, Mich., will be another speaker.

Recent interest in the use of alpha chymotrypsin in the management of cataract is the basis for another symposium, at which Drs. Derrick Vail, Chicago, and Olga Ferrer, Havana, Cuba, are scheduled to preside. Contributors to the symposium will include: Drs. Djordje Lukie M., University of the Andes, Merida, Venezuela; A. Benedict Rizzuti, Brooklyn, N.Y., and Louis J. Girard, Houston, Texas. In another session Dr. Joaquin Barraquer, Barcelona, Spain, will present a paper on this subject.

Cataract will also be considered in a round table session and in a symposium limited to surgery. In the first group the leaders announced are: Drs. Alfred E. Maumenee, Baltimore; John McLean, New York, and Jack Guyton, Detroit. Members of this panel will be: Drs. Ivo Correa Meyer, Porto Alegre, Brazil; Clovis Paiva, Recife, Brazil; Cyro de Rezende, São Paulo, Brazil; C. Espildora Luque, Santiago, Chile; Jesus Rhode, Caracas; J. M. Varas Samaniego, Quito, Ecuador, and Werther Duque Estrada, Rio de Janeiro.

The second group of speakers will include: Drs. Feliciano Palomino Dena, Mexico City; Hernando Garcia Gomez, Bogotá, Colombia; and Alberto Gormaz, Santiago, Chile.

Eye conditions that pose special problems in certain regions form the subject of a symposium under the chairmanship of Dr. Ida Mann of Australia, and Dr. Rodolfo Hernandez Miliani of Caracas. Dr. Mann has devoted many years of study to regional ophthalmology, having made extensive surveys of the prevalence of trachoma in Western Australia for the World Health Organization. She will discuss the value of such studies; other speakers will be: Drs. Guillermo Pico, Santurce, P.R., on causes of blindness in Puerto Rico, and J. Winston Duggan, Edmonton, Canada, on phlyctenulosis in Northwest Canada. Dr. Hernandez Miliani will report on strabismus as a public health problem in Venezuela.

Strabismus is also to be the subject of both a symposium and a round table discussion. Dr. Philip Knapp, New York, will lead the symposium, which will deal with incomitant horizontal strabismus; and Drs. John H. Dunnington, New York, and Rene Contardo, Chile, will be joint chairmen of the round table on surgery of strabismus. Participants in these sessions will include: Drs. William E. Krewson III, Philadelphia; Arthur Jampolsky, San Francisco; Frank D. Costenbader, Washington, D.C.; Edgardo Manzitti, Atilio Luis Norbis, Jorge Malbrán

and Enrique Malbrán, all of Buenos Aires; Francisco Vernaza, Bogotá, Columbia; Geraldo Queiroga, Belo Horizonte, Brazil, and Feliciano Palomino Dena, Mexico City.

Three speakers will deal with detachment of the retina: Drs. Robert J. Brockhurst, Boston; Bradley R. Straatsma, Los Angeles; and Alvaro Rodriguez Gonzales, Bogotá. In addition, Count Hermenegildo Arruga of Madrid, a leading authority on this subject, is expected to present in another session his experience with the equatorial ring operation in 120 cases, to be followed by several papers describing instruments for use in retinal surgery. Still another session will be devoted to diseases of the retina, with Dr. Jesus Rhode of Caracas, and Juan Arentsen Sauer, Santiago, Chile, as chairmen.

Glaucoma will be discussed in a round table on recent advances in treatment of the disease, led by Dr. Bernard Becker, St. Louis, and Jorge Valdeavellano, Lima, Peru. Speakers announced are: Drs. Hilton Rocha, Belo Horizonte, Brazil; Virgilio Victoria and J. M. Vila Ortiz, both of Rosario, Argentina; A. Torres Estrada, Mexico City, and Alberto Urrets Zavalia Hijo, Cordoba, Argentina.

At a subsequent session various aspects of glaucoma and its treatment will again be the topic of discussion. Drs. Carlos Carbino, Montevideo, Uruguay, and David Bitrán, Santiago, Chile, are scheduled to preside; and speakers will include: Drs. Kristjan Sveinsson, Reykjavik, Iceland; H. B. Escariz, Adrogue, Argentina; Francisco Rodriguez, Bogotá, Colombia, and Hernandez Miliani, Caracas.

Various advances in ocular surgery will be described in a session under the joint chairmanship of Dr. Baudilio Courtis, Buenos Aires, and Dr. Luis Sanchez Bulnes, Mexico City. Among the speakers will be Drs. R. Townley Paton, New York, on corneal transplantation; Rene Contardo, Santiago, Chile, on strabismus, cataract and the eyeball; Antonio de Almeida, Brazil, on glaucoma, refraction and lacrimal apparatus; Daniel Silva, Mexico City, on the lids and orbit. Dr. Sanchez Bulnes will discuss retinal detachment.

A series of 27 five-minute reports on progress in ophthalmic therapy will fill one morning session. Dr. Renato de Toledo, São Paulo, and Dr. Conrad Berens, New York, will be co-chairmen, and the list of participants includes:

Dr. Miguel Branly, Havana, Cuba, Vascular accidents in the retina.
Dr. Jorge Valdeavellano, Lima, Peru, Ophthalmic conditions of tuberculous origin
Dr. Aniceto Solares, Sucre, Bolivia, Conjunctivitis
Dr. Ayala Haedo, Asunción, Paraguay, Superficial keratitis
Dr. Julio A. Sicardi, Montevideo, Optic neuritis
Dr. Altino Ventura, Recife, Brazil, Secondary glaucoma
Dr. Colombo Spinola, Salvador, Brazil, Antibiotics in ophthalmology
Dr. Evaldo Campos, Rio de Janeiro, Analgesics, anesthetics and tranquilizers
Dr. Rubens Belfort Mattos, São Paulo, Granulomatous uveitis
Dr. Egon Kruger, Curitiba, Brazil, Diseases of the lacrimal apparatus

Dr. Conrad Berens, New York, will preside at a program on the prevention of blindness. Reporting on the current status of work in this field in their respective countries will be: Drs. Guillermo Pico, Santurce, Puerto Rico; Luis Sanchez Bulnes, Mexico City; Aniceto Solares, Sucre, Bolivia, and Baudilio Courtis, Buenos Aires.

Free Papers

Free papers will be presented in separate sessions. Among a large number of such presentations the following are included:

Dr. H. Saul Sugar, Detroit: The white limbus girdle of Vogt
Drs. Edward Grom and Carlos Soto, Caracas: The eye in art
Dr. Gilbert Baum, New York; The application of ultrasonography to ophthalmology
Dr. James E. Bennett, Cleveland: Surgical treatment of total xerophthalmia
Dr. Leo L. Mayer, Jackson, Miss.: Autopsy of the eye
Dr. Virgil J. Schwartz, Minneapolis: Congenital pterygium

Dr. F. Paez Allende, Santa Fe, Argentina: An operation of simple myopic astigmatism
Dr. John Simonton, New York: Surgical approach to the orbit
Dr. Jorge Malbrán, Buenos Aires: Surgical treatment of paralytic squint
Dr. Sidney A. Fox, New York: The use and misuse of irradiation in neoplasms of the lids
Dr. Antonio Ros, Mexico City: Evolution of the pathogenicity and therapeutics of trachoma
Dr. Louis H. Breffeilh, Shreveport, La.: The use of gamma globulin in the treatment of viral diseases of the eye
Dr. Joaquim Marinho de Queiroz, Belo Horizonte, Brazil: Schistosomiasis mansoni and eye manifestations

For those who plan to use electrical apparatus at the Congress, the Association is informed that the current in Caracas in 110 volts, 50 cycles, A.C.

Social Events

The Congress will open officially on Sunday, Jan. 31, with a formal luncheon at the Hotel Humboldt on Mount Avila, overlooking the city of Caracas. There will also be a formal closing banquet. For the visiting ladies trips are being planned to many points of interest in the city and its environs.

Dress in Caracas is the same as that worn in New York and Chicago. Formal evening wear will be needed only for the closing banquet. Gentlemen may wear white or black evening jackets. For all other affairs street and cocktail attire will be correct. Shorts and sport shirts are seen only on the beaches. Caracas has an altitude of about 3,000 ft., with an average temperature of 68 F. The temperature range is from 56 to 77 degrees.

Arrangements

Hotel and travel arrangements should be made without delay through Dr. William L. Benedict, 15 2d. St., S.W., Rochester, Minnesota, Chairman of the Committee on Transportation. Headquarters for the Congress will be at the Hotel Tamanaco.

New York Eye and Ear Infirmary Alumni Association.—The Annual Spring Meeting of the Alumni Association of the New York Eye and Ear Infirmary will take place April 4-7, 1960, at the Infirmary.

The meeting will be oriented towards pediatric ophthalmology. Symposia will be offered on: Nystagmus as a Pediatric Problem; Ocular Complications Attending Infectious Diseases of Childhood; Surgical Corrections of Congenital Deformities of Eye and Adnexa; Glaucoma and Cataracts in Pediatrics, and Office Management of the Pediatric Eye Patient. There will also be a closed circuit television demonstration of surgical procedures.

In addition, courses will be offered in the following subjects: Reading Problems; Pediatric Anesthesiology; Pleoptics; Congenital Syndromes; Pediatric Ophthalmic Therapeutics; Ocular Neoplasms and Their Treatment; Pediatric Neuro-Ophthalmologic Syndromes; Vertical Eye Muscles; Pediatric Ocular Allergy; Lacrimal Sacs, and Fundus Lesions of Childhood.

Additional Information may be obtained by writing to Dr. John R. Finlay, Secretary, Alumni Association, 218 2d Ave., New York 3.

Special Courses Offered.—The Mount Sinai Hospital of New York City together with Columbia University is giving the following courses: "The Use of the Schepens' Ophthalmoscope" from March 10 to March 24, 1960, and "Histopathology of the Eye" from April 6 to April 29, 1960. Complete information can be obtained from the Registrar for Medical Instruction, The Mount Sinai Hospital, 1 E. 100th St., New York 29.

Books

Synopsis of Ophthalmology. By William H. Havener. Price $6.75. Pp. 288, with 189 illustrations. C. V. Mosby Co., St. Louis 3, 1959.

This little book, intended for the general physician, covers most of the conditions which will be met in general practice and is a good practical guide to treatment.

It is good to see that the author utilizes space to define ophthalmologists, opticians, orthoptists, and optometrists for the general practitioner.

The chief criticism of the book is its cost, which seems exhorbitant for its size.

Headache; Diagnosis and Treatment. By Arnold P. Friedman and H. Houston Merritt. Price $8.00. Pp. 401, illustrated. F. A. Davis Co., 1914 Cherry St., Philadelphia 3, 1959.

This symposium on headache consists of 13 chapters contributed by well-known specialists in the field. A review of the mechanisms of headache by Kunkel describes the pathologic physiology producing this type of pain, and the methods by which the physician can diagnose the various types of headache.

A chapter by Friedman and Merritt, the editors, follows on the principles of pharmacotherapy, including the choice of drugs, indications for their use, and contraindications. The eye as a source of headache has been covered by Max Chamlin. The editors have also written a separate chapter on migraine, which will be of value to ophthalmologists.

This book should be in every ophthalmological library.

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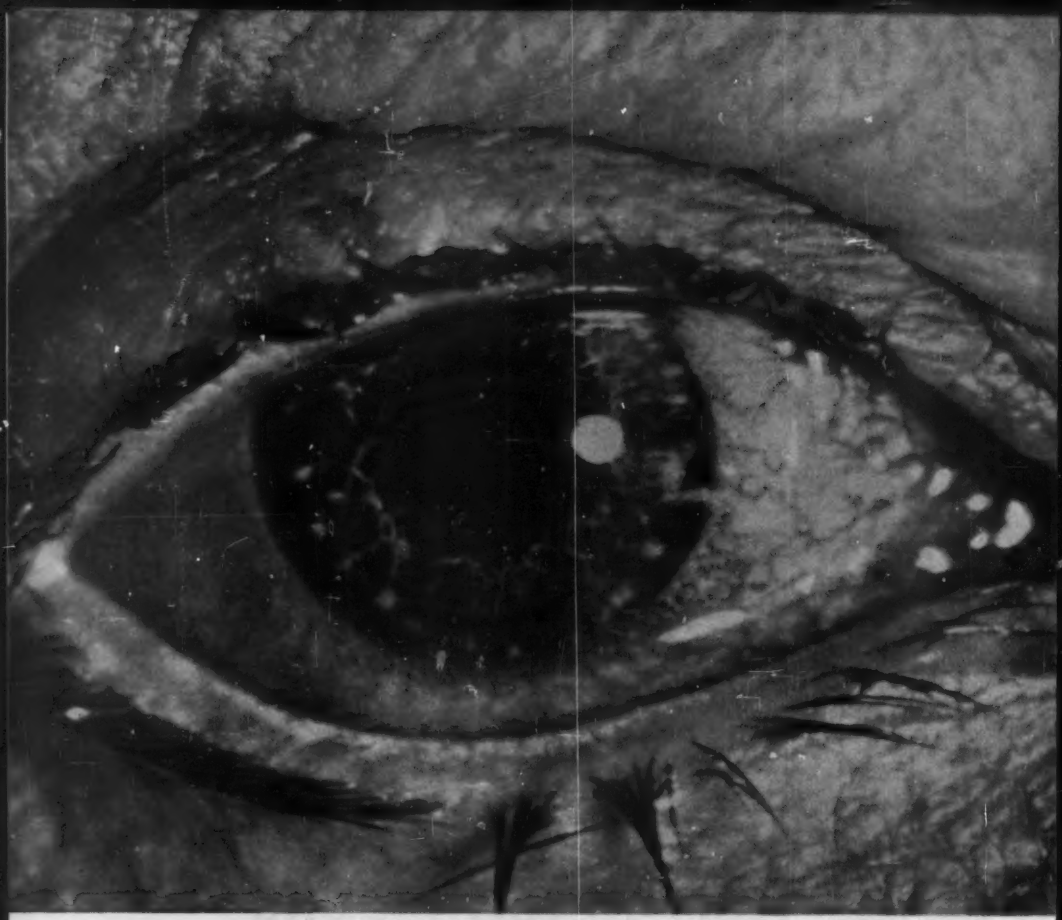

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1. Gordon, D. M.: Scientific Exhibit, American Medical Association, Annual Meeting, San Francisco, 1958.

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dditional information is available to physicians on request.

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Product	Steroid Concentration	active ingredients: Dexamethasone 21-Phosphate (as the disodium salt)	active ingredients: Neomycin Sulfate	Supplied
NeoDECADRON Ophthalmic Solution	0.1%	1 mg./cc.	5 mg./cc. (equivalent to 3.5 mg. neomycin base)	5 cc. (¼ oz.) sterile dropper-bottles
DECADRON Phosphate Ophthalmic Solution	0.1%	1 mg./cc.	—	5 cc. (¼ oz.) sterile dropper-bottles
NeoDECADRON Ophthalmic Ointment	0.05%	0.5 mg./Gm.	5 mg./Gm. (equivalent to 3.5 mg. neomycin base)	3.5 Gm. (⅛ oz.) tubes
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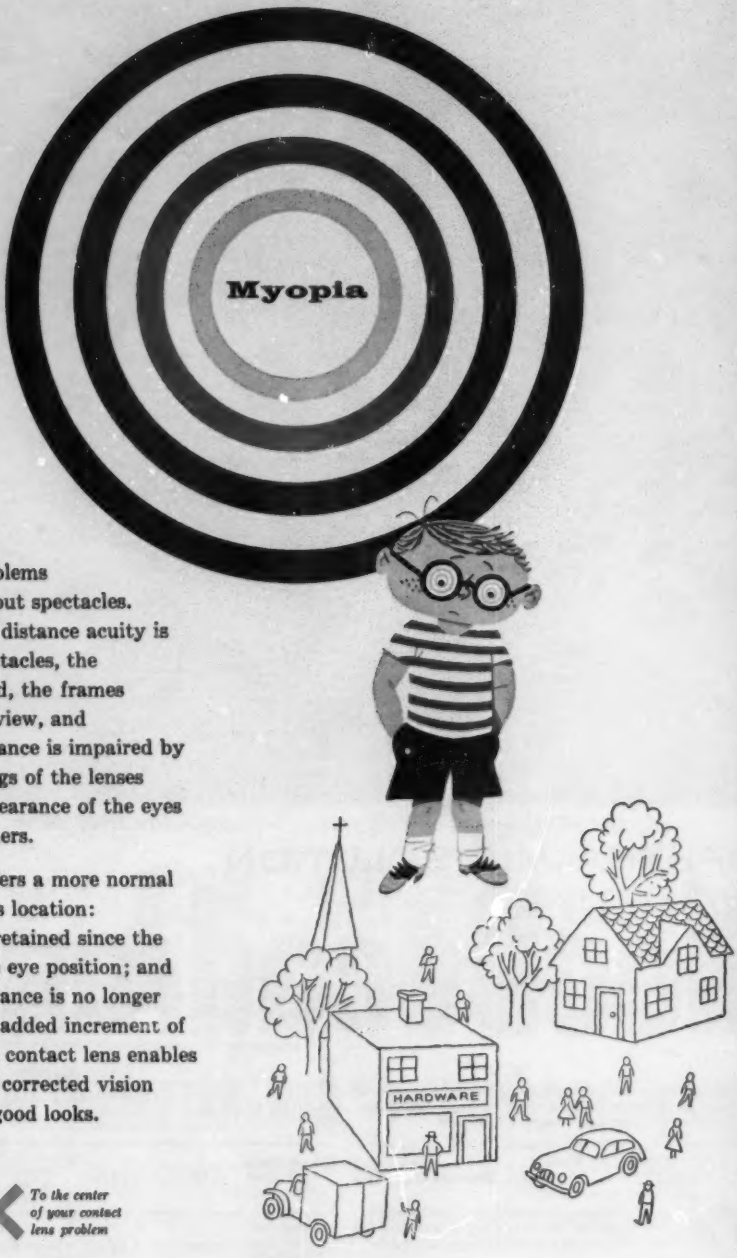

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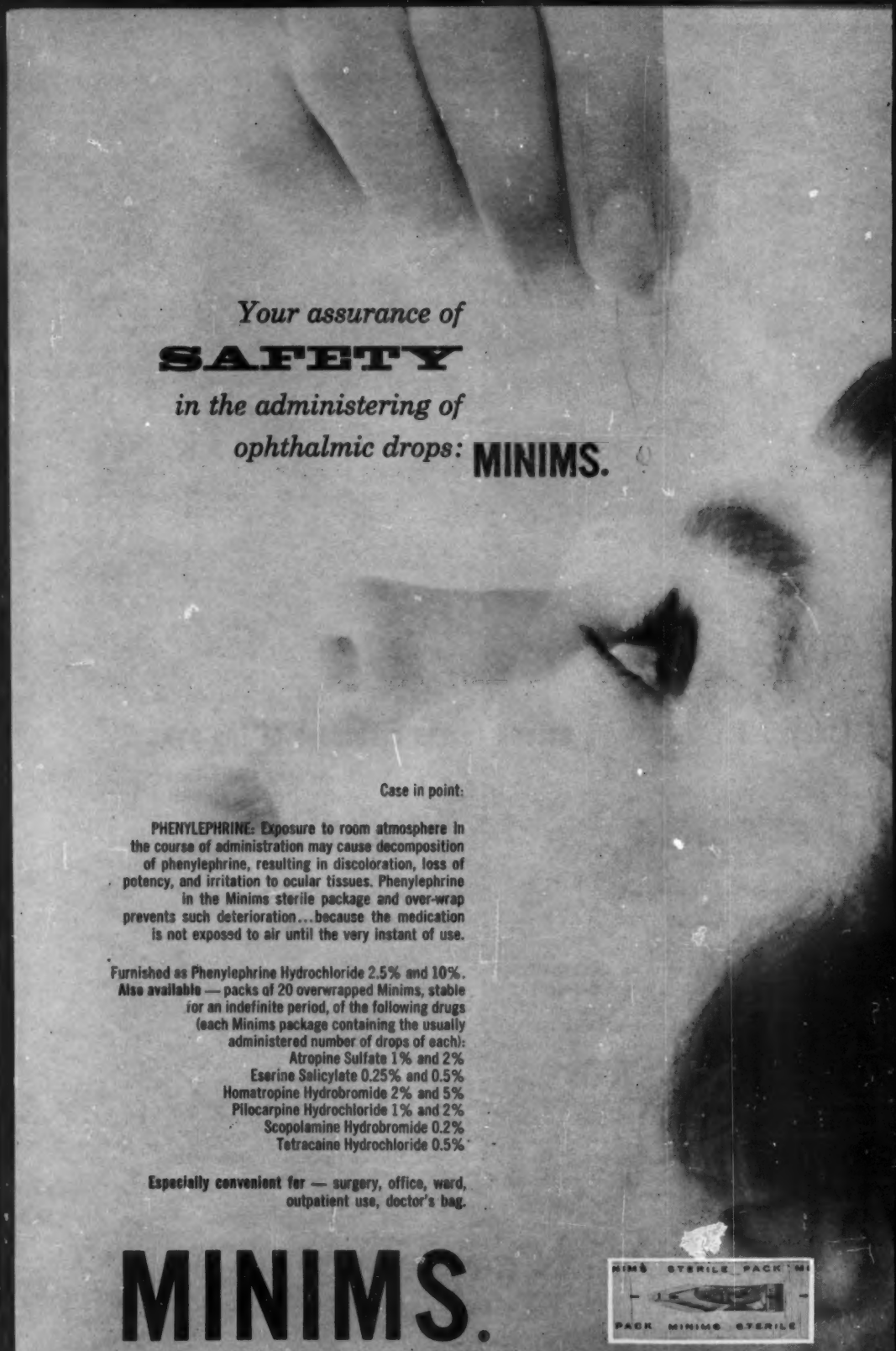

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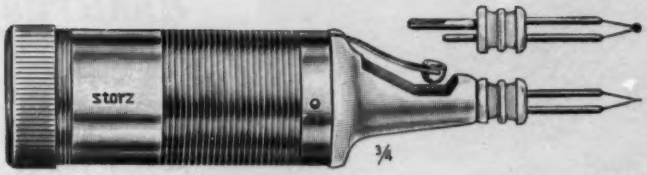

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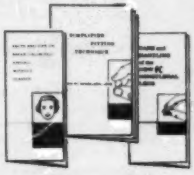

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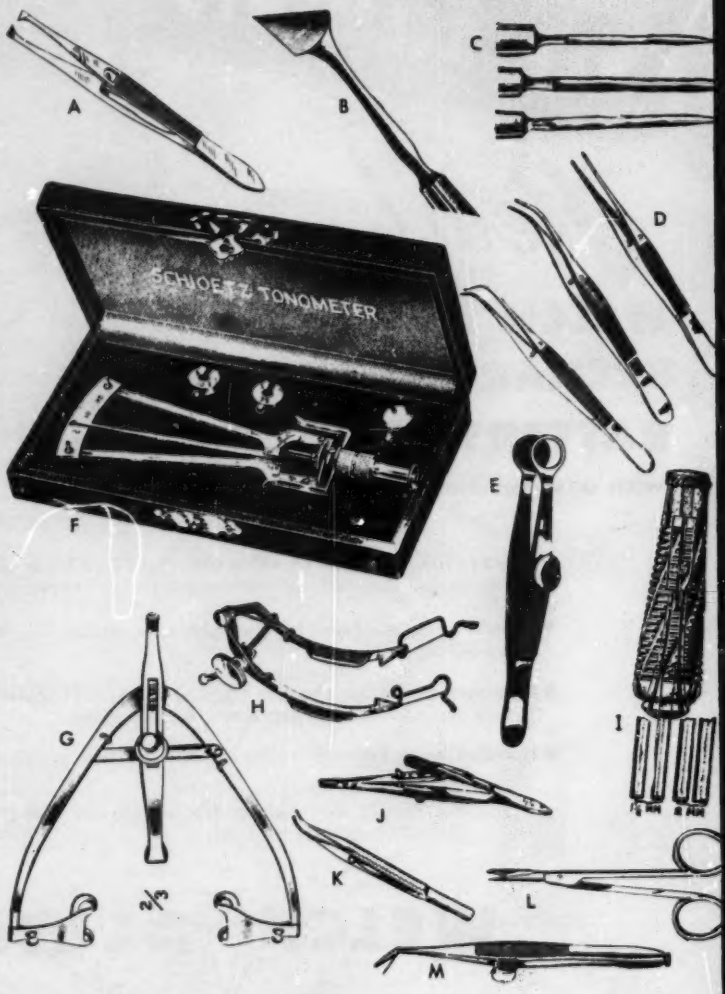

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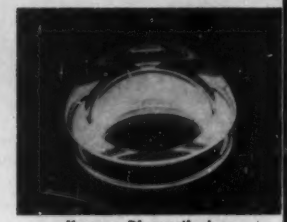

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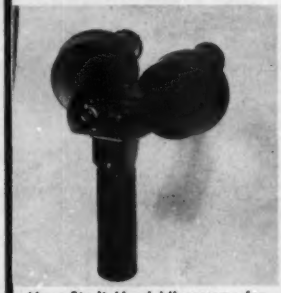

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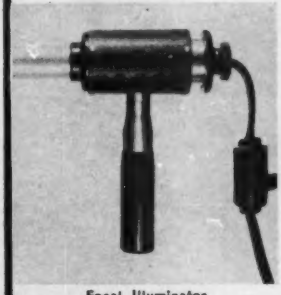

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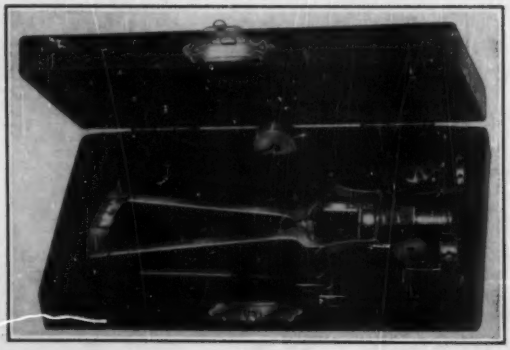

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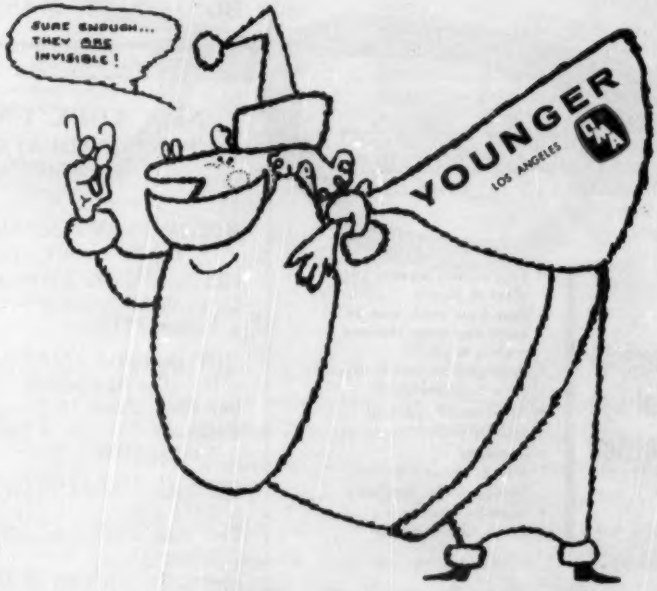

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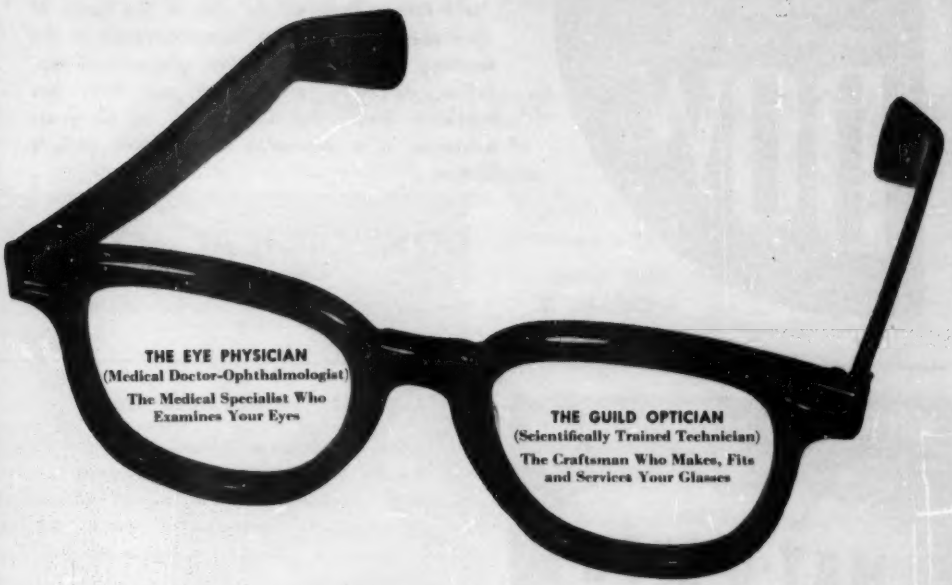
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The eyes should be placed in a sterile bottle, packaged with the history and findings, and shipped as quickly as possible. Please send specimens special delivery, collect, and mark the package "Fresh Tissue Specimen—Rush." Do not freeze or use preservatives of any kind.

A report of isolations of organisms and pathologic findings, including a slide, will be sent to the contributor. Credit will be given in any resulting publications if desired.

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Send eyes to

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antibacterial
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Supplied: 5 cc. dropper bottle.

METIMYD,® brand of prednisolone acetate and sulfacetamide sodium.

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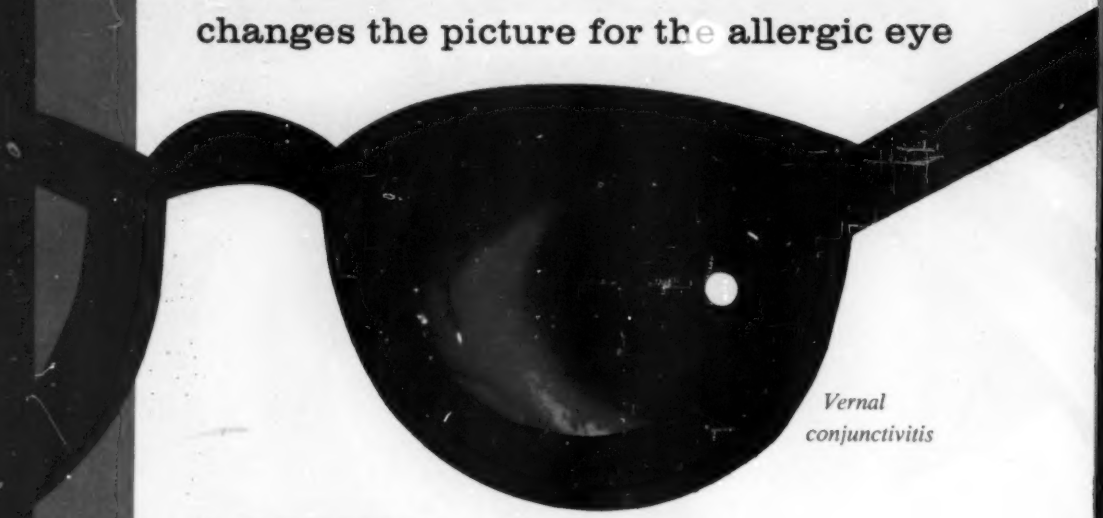
changes the picture for the allergic eye



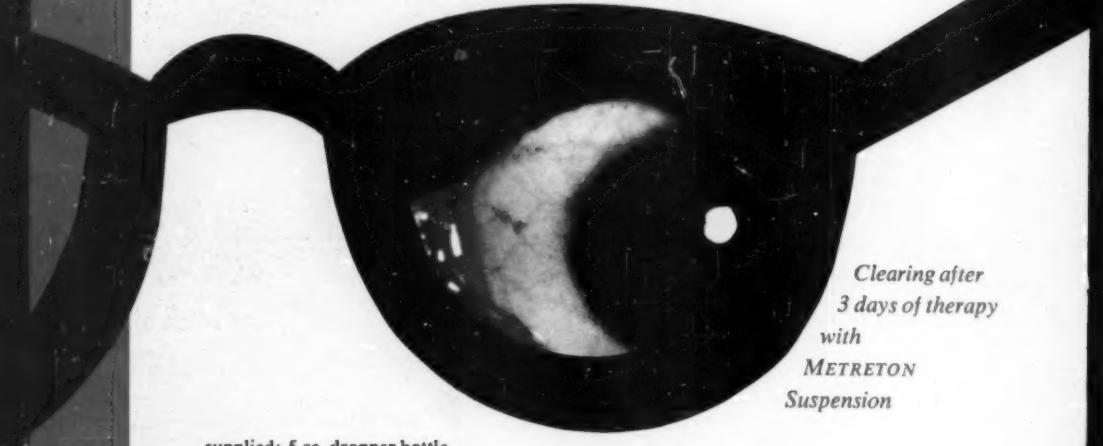
Vernal conjunctivitis

METRETON

Ophthalmic Suspension

combining METICORTELONE and CHLOR-TRIMETON

in vernal conjunctivitis / episcleritis
allergic blepharitis / ocular involvement of hay fever
allergic conjunctivitis / other ocular allergies



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supplied: 5 cc. dropper bottle.

METRETON,® brand of corticoid-antihistamine compound.
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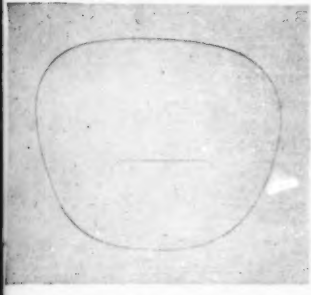
SCHERING CORPORATION • BLOOMFIELD, NEW JERSEY

MS-J-119

"SECRET" SEGMENT "SECRET" SEGMENT ????

Performance, Identification,
appearance, too!

UNIVIS I.S./22
*IDENTIFIED SEGMENT



Note the perfectly straight-top segment line and rounded corners in the unretouched photograph.

Not that the segment is invisible. It isn't. But it's extremely inconspicuous because of very thin segment line, *shorter* segment line, rounded corners and perfect fusion.

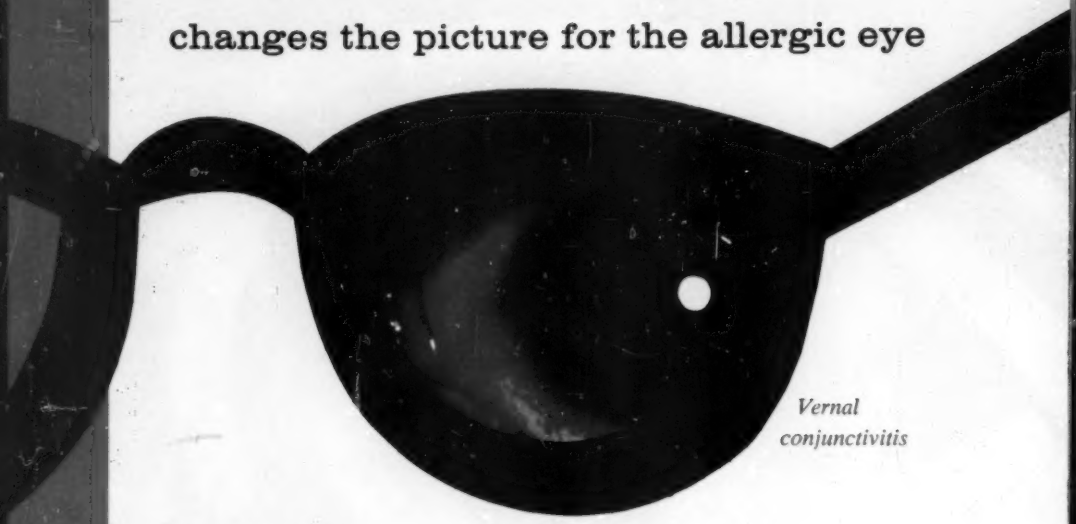
Who wants "secret" segments? Many bifocal wearers, many of those who prescribe and fit them. Cosmetic excellence is one reason why new Univis I.S./22 is destined to become one of America's most popular lenses.

What else? Minimum segment jump with optical center only 4 mm. below segment line. Perfectly straight-top segment to eliminate patient confusion and for maximum accuracy of compounded Rx's. Perfectly matched segments and complete freedom from cylinder and aberration through the precise control of Univis-designed 8-zone low temperature furnace.

Identification? *Just look for the segment shape.*

THE UNIVIS LENS COMPANY / Dayton, Ohio

changes the picture for the allergic eye



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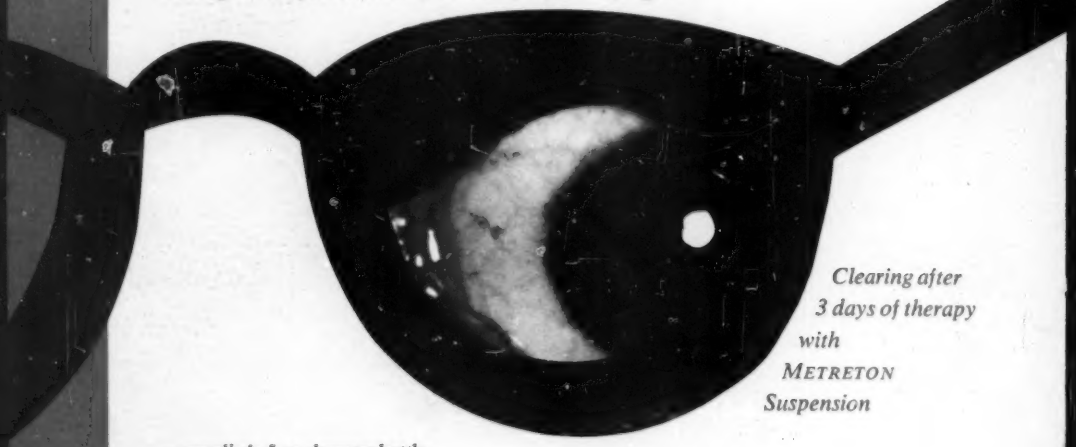

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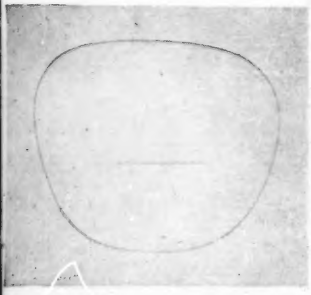

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